STRATEGIES FOR OPTIMIZING PERCUTANEOUS ABSORPTION

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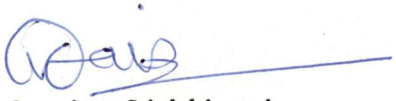
This thesis contains no material which has been accepted for the award of any other degree or diploma in any University or College.

To best of my knowledge and belief, this thesis contains no material previously published or written by another person, except when due reference is made in the text of the thesis.



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SUMMARY

Studies were carried out on the *in vitro* permeation of steroids and weak electrolytes through the human stratum corneum. Diffusion and compartmental models were used to describe the mechanism of permeation of the ionised and unionised moeities of the above drugs. The effect of iontophoresis and the pH of aqueous vehicles on the rate and extent of permeation of weak electrolytes was also investigated. The relative contribution of epidermal and dermal transport of methotrexate and steroids was examined in an attempt to define the concentration of these drugs likely to be found in the viable epidermis.

The results obtained using mathematical models suggest that the main route of skin penetration of both steroids and weak electrolytes through the excised human stratum corneum is more likely to be through the "intracellular" route than through the "shunts". The present work using diffusion and compartmental models suggests that both ionised and unionised species of weak electrolytes can permeate the excised human stratum corneum. There is evidence to indicate the presence of a "pH-shift" for some weak electrolytes and that the ionised species of weak electrolytes form an ion pair with counter ions present in a vehicle. The formation of an ion pair and its ability to partition into the lipid phase with relative ease also indicates that ionised species of weak electrolytes are likely to penetrate the excised human stratum corneum through the "intracellular"

route.

In the absence of iontophoresis, the rate of penetration of weak electrolytes was greatest at a pH value where the substance was mainly in the unionised form; iontophoresis was most effective at a pH value where the weak electrolyte was mainly in the ionised form. It is postulated that during the application of iontophoresis the "shunt" pathway could be the major route of skin penetration.

The rate of dermal disappearance of methotrexate and steroids was found to depend on both the dermal blood supply and the rate of diffusion into the dermis. It was found that the epidermal penetration rate and dermal clearance play a significant role in determining the concentration of a drug likely to be achieved in the viable epidermis. The present results suggest that high dermal clearance may reduce the efficacy of topically applied medication.

GLOSSARY OF SYMBOLS

C	concentration of solute
Cl	clearance
C_{ss}	concentration in the viable epidermis
C_v	donor concentration
%CV	coefficient of variation
D	diffusion coefficient
e	epidermal transport (intracellular route)
h	thickness of the membrane
i	ionised species
J	flux i.e. rate of penetration in amount/area
J_{ss}	steady state flux in amount/area/time
K	solute-membrane partition coefficient
K'	octanol-water partition coefficient
K''	octanol-water partition coefficient based on rubidium or chloride counts
K_a	ionisation (dissociation) constant
k	first order rate constant
k'	first order rate constant for ionised species
k''	first order rate constant for unionised species
k_p	permeability coefficient
L	thickness of the membrane
M	cumulative amount of solute penetrating the membrane/area
m	inert membrane
N	number of data points
n	number of data points

r	correlation coefficient
R	partition coefficient
s	shunts (intercellular and transfollicular routes)
t	time
t_L	lag time
u	unionised species
V_{dss}	apparent volume of distribution at steady state

CHAPTER 1

INTRODUCTION

1.1 Historical

Since medieval times physicians have tried to treat human ailments by applying medicaments to the skin. A variety of ointments, pastes, plasters and complex inunctions were used in attempts to cure every conceivable disease. In the late nineteenth century the emphasis was changed to the use of tablets, pills and mixtures because it was realized that no matter how hard a medicament was rubbed over the entire body there was no therapeutic response whereas with oral drugs a response, even if it was an adverse reaction, was actually visible (Kligman 1983).

The extreme view held in the late nineteenth century that the skin was totally impermeable did not hold for long as physicians were more or less successfully treating syphilis by rubbing salts of mercury over the skin. It was shown later that lipid soluble agents were more likely to penetrate the skin than those which were water soluble, and that polar electrolytes and ionised salts penetrated very poorly, a view still held for a number of substances (Kligman 1983). This view on the permeability of ionised species follows from the pH-partition hypothesis, which states that only the unionised forms of drugs are able to pass through biological membranes in significant amounts (Shore et al 1957).

1.2 Modern scene

The pH-partition hypothesis (Shore et al 1957) is based on the

assumption that the predominantly lipophilic nature of biological membranes, for example the stratum corneum and the intestinal tract, would act as barriers to the ionised species. The contribution of pores (shunts) present in these membranes to the penetration of ionised species is disputable (sections 2.2.1 and 2.3.1.7).

Wagner (1975) reviewed the modifications to the pH-partition hypothesis, suggested by a number of workers including Suzuki et al (1970), Ho and Higuchi (1971), Ho et al (1972) and, Wagner and Sedman (1973). Suzuki et al (1970), Ho and Higuchi (1971), and Ho et al (1972) had proposed that the absorption of ionisable drugs could be rate limited in the gastrointestinal tract by the presence of an aqueous diffusion layer on the lumen side of the tract and it was more more likely that the transport of the ionic species of the drug occurs through water filled pores of the gastrointestinal membrane. On the other hand the model proposed by Wagner and Sedman (1973) takes into consideration the partitioning of ionic species into the membrane and does not incorporate the concept of transport through the pores. However, a number of in vitro studies (with isolated intestinal membrane) have shown that both ionised and unionised species of a drug can permeate a lipid membrane (Nogami et al 1962; Notari 1982).

1.3 Topical dosage forms

Skin, as a continuous membrane, is an essential organ of the human body (Figure 1.1). The main function of the skin is to protect the internal organs and tissues from physical or environmental attack; it also prevents the loss of essential

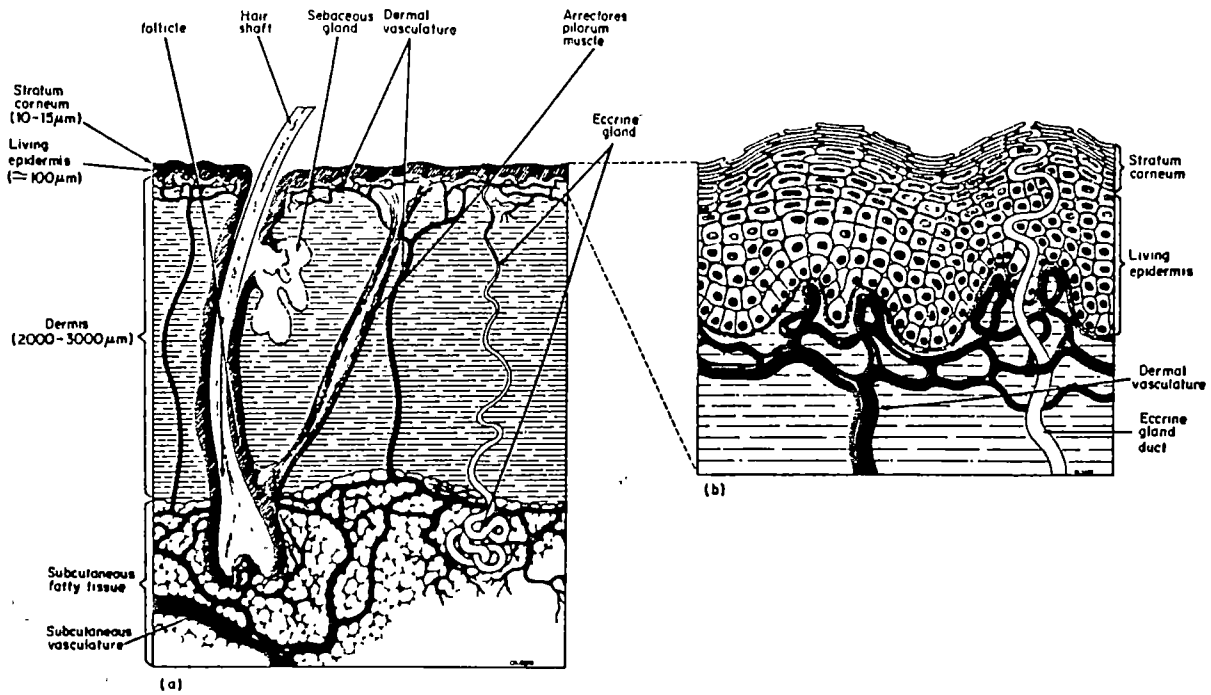


Figure 1.1 Schematic cross section of the skin. The full thickness is shown on the left - (a) and expansion of the epidermis is shown on the right - (b) (Flynn 1979).

ingredients and fluids from within the body to the environment.

As the skin is the most accessible tissue of the body it is continually challenged, chemically and biologically, and it may become damaged or infected by a number of micro-organisms. The skin is capable of repairing itself after infliction of any minor damage; when the damage or infection is more severe, the use of drugs becomes imperative. A number of inflammatory conditions arising from internal disorders may also require the use of topical drugs (Katz and Poulsen 1971; Figure 1.2).

A topical dosage form can only be effective if the active ingredient is capable of reaching the site of the skin disorder (Figure 1.2). This can only be achieved by controlling the physicochemical properties of the dosage form (section 2.3.1) to ensure the optimum delivery of the drug to the site of the skin disorder or infection.

The existence of rich blood capillaries in the skin (Figure 1.1) also allows it to be used as a site for the administration of drug(s) intended to produce systemic effects (Figure 1.2). Although this was realized in the 1930's (Macht 1938) it was not until the early 70's, as reported in The Pharmaceutical Journal (Anon 1970), that research was undertaken to develop a dosage form which could be used to administer drug(s) through the skin for systemic effects. The research workers at Alza corporation (California, USA) later developed and successfully tested a dosage form, called the transdermal therapeutic system (TTS), containing hyoscine (scopolamine) incorporated in a vehicle, which was capable of preventing motion sickness (Zaffaroni 1971).

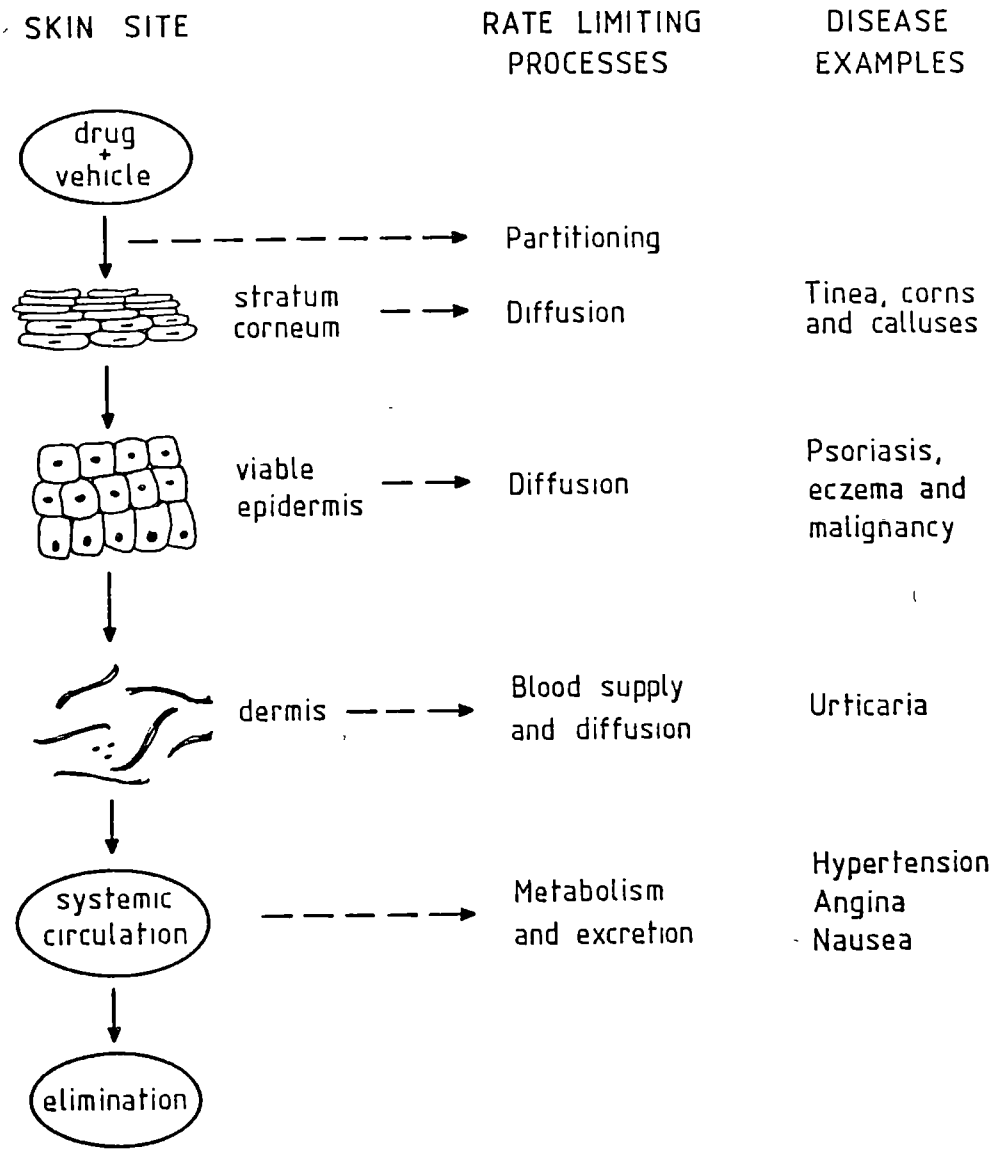


Figure 1.2 Rate limiting steps in the absorption of drugs and sites of action required for topical medication.

This has now been followed by the introduction of TTS's containing each of nitroglycerin and clonidine. The use of transdermal therapeutic systems has a number of potential advantages over the more conventional oral dosage forms. A transdermal system eliminates the variables affecting gastrointestinal absorption of oral dosage forms and it also reduces drug metabolism and side effects which are generally associated with oral dosage forms (Heilmann 1978).

Although the use of transdermal systems has revolutionized the whole concept of systemic drug delivery through the skin, its use is limited to selected drugs. The therapeutic effect of any drug is dependent upon the concentration of the drug that can be attained at the site of disorder (Schaefer et al 1982; Figure 1.2). Therefore the topical administration of a drug(s) for local effect in the damaged or infected parts of the stratum corneum or viable epidermis (Figure 1.2) is also an important area of investigation for different classes of drugs.

1.4 Optimization of percutaneous absorption

The classical approach to optimizing the rate of percutaneous absorption is through the manipulation of the physicochemical factors of the topical dosage form (section 2.3.1). The effect of physiological factors (section 2.3.2) must also be considered during the optimization of the rate of penetration of a particular drug(s). Some of the more important physicochemical factors are discussed below.

Vehicles

The concentration and solubility of a drug in a vehicle (sections 2.3.1.3 and 2.3.1.4) are important considerations in the choice of an ideal vehicle. An optimum vehicle would keep almost all the drug in solution while remaining nearly saturated with the drug (Flynn 1979). This would enable the topical dosage form to release the maximum amount of the drug to the surface of the skin, which in turn gives a higher rate of penetration than that would occur with a lower concentration of the drug.

Chemical structure

A drug molecule may have to be chemically modified to optimize its penetrating capabilities in order to obtain the necessary therapeutic effect. Some corticosteroids show an increase in effectiveness following chemical modification; for example if 0.05% of betamethasone 21-valerate was used instead of 0.5% or 1% of hydrocortisone a significant increase in effectiveness was realised. This increase in effectiveness was reported to be due to the high lipid solubility of betamethasone 21-valerate relative to that of hydrocortisone (Katz and Shaikh 1965; Flynn 1979).

Other factors

There are several other physicochemical factors (section 2.3.1) which can influence the optimum rate of drug delivery from a topical dosage form. Most of these factors, for example degree of hydration, the presence of penetration enhancers, the presence of occlusive vehicles and/or dressings induce changes in the

physicochemical properties of the stratum corneum (Flynn 1979) which may change the barrier properties of the stratum corneum resulting in an increased rate of penetration by the active ingredient.

1.5 Present work

The main purpose of much of the research in percutaneous absorption has been to quantitatively predict skin permeability in terms of the relevant physical and chemical properties of the drug (Scheuplein and Blank 1971; section 2.3). Kligman (1983) has suggested that the modern foundation for understanding the principles and the mechanisms of the percutaneous absorption of drugs can be built on two experimental pillars: (1) measurement of flux *in vitro* using excised skin mounted in a permeation cell and (2) the use of radioactive materials or other sensitive analytical techniques (section 2.4), which will allow accurate *in vivo* and *in vitro* measurements. These methods have formed the experimental basis of the present study on steroids and weak electrolytes using *in vitro* permeation cells.

1.5.1 Percutaneous absorption of non-electrolytes and weak electrolytes

Non-electrolytes (e.g. steroids) and weak electroltyes are compounds which are widely used in topical products for their anti-inflammatory, anti-pruritic, anti-allergic, anti-bacterial, anti-psoriatic, anaesthetic and/or analgesic effects. Numerous *in vitro* and *in vivo* studies have been carried out on both human and animal models using these compounds. These studies have been

reviewed by Tregear (1966b); Katz and Poulsen (1971); Scheuplein and Blank (1971); Mauvais-Jarvis et al (1980); Schaefer et al (1982) and Barry (1983).

The literature contains a wealth of information on various aspects of steroids and their use in topical dosage forms (Chapter 2). Much of the published in vitro work relates to the effect of physicochemical factors (section 2.3.1) on the rate of penetration of these compounds. Limited work by Scheuplein et al (1969) and others reported in the reviews by Schaefer et al (1982) and Barry (1983), has been done on the mechanism of the penetration of steroids.

The salicylates and lignocaine are probably the most important weak electrolytes used in topical dosage forms. Methotrexate has been subjected to numerous in vivo and in vitro investigations in order to identify the reason for its ineffectiveness in topical therapy (Schaefer et al 1982). Much in vitro work has been done on the influence of formulation factors of weak electrolytes and steroids (section 2.3.1). Various mechanisms of penetration for polar and non polar substances (section 2.2) have also been suggested by Scheuplein (1966,1967,1972), Higuchi (1977) and Roberts et al (1978).

There has apparently been little work, in which the emphasis has been placed on the effect of the degree of ionisation of the substance on the rate of its penetration through human skin, done on weak electrolytes (section 2.3.1.7). Such problems have however been investigated for salicylates (Marcus et al 1970); ephedrine, chlorpheniramine and scopolamine (Michaels et al 1975)

and methotrexate (Wallace et al 1978). Michaels et al (1975) used a mathematical model to predict that the unionised species generally achieve a greater degree of permeation than do the ionised species. More recently however, Swarbrick et al (1984) have shown that chromone-2-carboxylic acids permeated excised human skin both as unionised and ionised species. Therefore despite previous work on steroids and weak electrolytes it is apparent that there are still a number of aspects of the penetration kinetics of these compounds which are not fully understood.

1.5.2 Purposes of the present work

Steroids and weak electrolytes have been chosen as model compounds in this work because they represent a cross section of the drugs which are used in topical dosage forms and have a wide range of physicochemical properties.

The main objective of the present study has been to develop strategies by which the percutaneous penetration of topically applied substances may be optimized. These strategies are based on the interplay between the molecular structure of the substance, the physiology of the skin and the conditions of application. Experiments were carried out to determine the in vitro rate of permeation of a number of steroids, with varying molecular structure, through the human stratum corneum. The role of pH and the effect of iontophoresis on the in vitro rate of permeation of weak electrolytes, with different molecular structures, through the human stratum corneum was also investigated. The overall intention was to describe the mechanism

of permeation using mathematical models, of the ionised and unionised moeities of the above drugs with a view to optimizing the rate of penetration through the human stratum corneum.

The relative importance of the epidermal and dermal barriers, and the role of the dermis and the cutaneous blood supply in controlling the rate of percutaneous penetration of methotrexate and steroids, was also investigated as the concentration of the substance in the viable epidermis is determined not only by epidermal penetration but also by dermal clearance.

It was considered that the successful completion of the work could lead to suggestions for developing strategies to control and optimize the bioavailability of drugs, applied to the skin, for either local or systemic effect.

CHAPTER 2

PERCUTANEOUS ABSORPTION OF DRUGS

A topical product may be applied to the skin for a local effect, an effect in the viable layers of the skin or a systemic effect (Figures 1.1 and 1.2). The process of percutaneous absorption can be defined as the mass movement of substances from the skin surface to the general circulation. It includes penetration through the stratum corneum, diffusion through each layer of the skin and finally the uptake by the skin's microcirculation (Figures 1.2 and 2.1). The occurrence of percutaneous absorption is attributed to the passive diffusion of the drug from a carrier or a vehicle on the surface tissues of the skin to reach the systemic circulation in the dermis (Scheuplein 1978a; Schaefer et al 1982).

The permeation of drugs through biological or synthetic membranes may occur due to passive, active or facilitated transport (Flynn et al 1974). The transport of drugs through the skin mainly occurs by a passive diffusion process (Katz and Poulsen 1971). The term "transport" used throughout the present work refers to the passive diffusion of drugs through human or animal skin.

Research in the field of percutaneous absorption has gained considerable prominence in recent years through the development of TTS's and the related interest in the design and the use of topical products (Zafforani 1971; Heilmann 1978; Shaw and Chandrasekaran 1978; Black 1982, 1983; Macek 1983; Holford and Paton 1984).

In order to successfully utilise the phenomenon of percutaneous

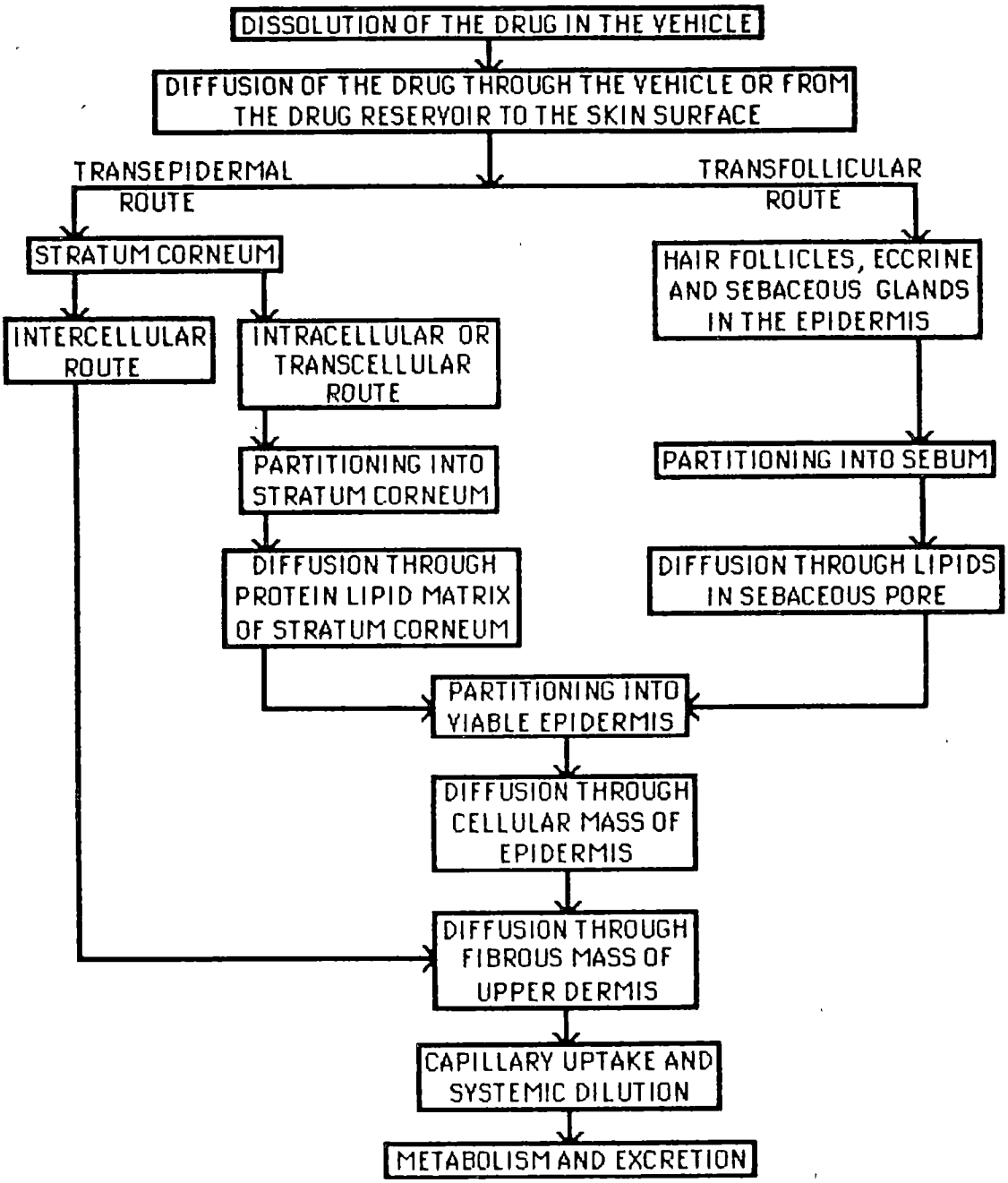


Figure 2.1 Schematic representation of percutaneous absorption (modified from: Katz and Poulsen 1971; Flynn 1979; Barry 1983).

absorption in drug delivery it is necessary to understand the anatomy of the skin, its physiological function, its physical and chemical composition and its biomechanical properties.

2.1 Anatomy

The human skin is a heterogenous membrane which consists of three distinct layers, the epidermis, the dermis and the subcutaneous fat. These layers can be further distinguished as shown in Figure 1.1 by dividing the epidermis into two parts. Thus a suitable classification is as follows:

2.1.1 The non-viable epidermis or the stratum corneum

The stratum corneum (horny layer or cornified layer) is the outermost layer of the skin and is recognized as the principal barrier to water loss and entry of noxious environmental agents. It is due to the effectiveness of the stratum corneum in preventing water loss from the inner layers of the skin that all other normal tissues are able to maintain dynamic equilibrium with the aqueous environment within the human body (Blank and Scheuplein 1964; Idson 1978).

The stratum corneum, which weighs about 1.5 g cm^{-1} in the dry state, is multicellular, metabolically inactive dead tissue derived from previously viable epidermal cells. The thickness of non-hydrated stratum corneum over most of the body is approximately 10-50 micrometers; however on the palms of the hand and soles of the feet the thickness may be of several hundred micrometers (Scheuplein and Blank 1971; Wilkes et al 1973). Plewig et al (1983) used the vertical dimensions of single

corneocytes to determine the thickness of the stratum corneum and concluded that the total thickness of the stratum corneum is around 6 micrometers over most of the body.

The stratum corneum is continually shedded and it is replaced by the migration of the germinal cells towards the surface. This is a complex process in which the germinal cells flatten, dehydrate and undergo intercellular polymerization (keratin formation), so that when the stratum corneum evolves it is a dense, keratin filled and metabollically inactive layer consisting of alpha-keratin and beta-keratin (Tingstad et al 1958; Brody 1966; Wilkes et al 1973; Montagna and Parakkal 1974).

Keratin present in the cells of the stratum corneum is a fibrous protein which is poor in sulphur, forming a filamentous network which assures cohesion, flexibility and recovery. The unique properties of stability, insolubility and resistance observed in the stratum corneum are due to the thick cell membrane and cell matrix which consists of amorphous proteins rich in sulphur content and lipids with many disulphide linkages (Matoltsy and Parakkall 1967). The stratum corneum is described as the only rate limiting barrier of the skin with regard to the viable epidermis and dermis (Idson 1978; Schaefer et al 1982).

2.1.2 The viable epidermis

The viable epidermis includes stratum germinativum (germinal layer or proliferative layer or basement layer), above which are the stratum spinosum (prickle cells), stratum granulosum (granular layer) and the stratum lucidum (lucid layer or the

keratogenous zone). The stratum spinosum and stratum germinativum are together called the malphigian layer.

The viable epidermis is different from the stratum corneum; it is physiologically more closely akin to other living cellular tissues. The viable epidermis is an aqueous solution of protein encapsulated into cellular compartments by thin cell membranes which are fused together by tonofibrils. The viable epidermis has a density near to that of water (Flynn 1979).

Histologically, the viable epidermis has four distinct layers between the stratum corneum and the dermis. The germinal layer above the dermis undergoes cell division producing an outward displacement of the cells towards the surface. These germinal cells prepare to die as soon as the displacement begins by synthesizing keratin. Melanin produced by melanocytes is necessary to give colour to the skin; these are present at the junction of the germinal layer and the dermis. As the germinal layer moves upwards it changes its shape into a more rounded form and appears as stratum spinosum. After the germinal layer has risen 12-15 layers above its point of origin, it becomes flattened and takes the form of stratum granulosum, which is said to be the last living cellular stage of the viable epidermis (Montagna 1964; Blank and Scheuplein 1964; Katz and Poulsen 1971).

Just before the formation of the stratum corneum, there is an increase in stages of nuclei disintegration and keratinization in the stratum lucidum, thus the name keratogenous zone. There is an intense morphological transformation with biochemical and

physiological activity to produce a sulphahydryl rich protein matrix, which will move upwards to eventually form the stratum corneum (Kligmann 1964; Winkelmann 1969).

Langerhan's cells are also reported to be present in the viable epidermis. It has been suggested that these cells may be involved in the immune response of the skin by binding the antigens, which probably modifies them, and transports them to the lymph nodes for release of leucocytes (white blood corpuscles) for lymphocyte activation (Barry 1983).

2.1.3 The dermis or corium

This is an important part of the skin which is about 0.2 cm to 0.3 cm thick and is made up of semi-gel matrix of micropolysaccharides. This matrix is a dense network of structural protein fibres mainly collagen, elastin and reticulum. The dermis contains the reticular layer which is the main structural layer of the skin and the papillary (Flynn 1979).

The dermis is penetrated by hair follicles and sweat glands. It has a very rich blood supply (blood flow is about 0.05 ml/min per cubic centimeter at approximately 23^{o}C) and provides the entire body with temperature, pressure and pain regulating mechanisms (Rothman 1954; Scheuplein and Blank 1971; Katz and Poulsen 1971; Flynn 1979).

The lymphatic circulatory system present in the dermis also performs some of the functions associated with nutrition, mobilization of defense mechanisms and waste removal. An important function of the skin circulatory system is its ability

to "switch on and off" in response to the presence of vasodilators and vasoconstrictors respectively (Stougton 1962; Mckenzie 1962; Woodburne 1965; Katz and Poulsen 1971).

2.1.4 The subcutaneous fatty tissue

Here the cells contain large quantities of fat, making the cytoplasm lipoidal in character. The collagen between the fat cells provides the linkage of epidermis and the dermis with the underlying structures of the skin. The main function of subcutaneous fatty tissue apart from storing calories, is to act as a heat insulator and a shock absorber (Katz and Poulsen 1971).

2.1.5 The skin appendages

The skin surface has several types of appendages; these include hair follicles with the sebaceous glands, eccrine sweat glands, apocrine sweat glands and the nails. Katz and Poulsen (1971) have given a detailed account of these appendages and have concluded that all these glands have different functions depending upon the part of the body in which they are found (Table 2.1) as they are anatomically quite dissimilar to each other.

2.2 Kinetics of percutaneous absorption

Kinetics can be defined as the rate of movement of a substance from one site to another. The penetration kinetics of any substance applied to the skin can be divided in two phases. The first phase is called the transient diffusion or non-steady state while the second phase or steady state is achieved once an equilibrium in the rate of entry and the rate of exit of a drug

Table 2.1 A comparison of secretory glands of the skin [from Katz and Poulsen 1971]

	Sebaceous	Eccrine	Apocrine
Distribution	Over entire body	Over entire body	Axillae, nipples, ano-genital
Average #/cm^2	15-100	100-200	Variable
Fractional area . .	2.7×10^{-3}	8×10^{-3} to 3×10^{-5}	Variable
Exit	Hair follicle	Own pore	Hair follicle
Secretion	Sebum	Dilute saline	"Milk" protein, lipo-proteins, lipid
Function	Lubrication	Cooling	Vestigial secondary sex gland?
Stimulated by . . .	Heat (minor)	Heat, cholinergic	Heat
Drug response		Acetylcholine and	
stimulatory. . . .	--	Pilocarpine	--
inhibitory	--	Anticholinergic	Anticholinergic
Control	Hormonal	Sympathetic nerves	Sympathetic nerves

through the layers of the skin is established (Scheuplein 1967; section 2.5).

Skin is a heterogenous membrane and any drug molecule which is required to give systemic effect will have to penetrate through all the layers of the skin (Figures 1.2 and 2.1). To understand the transport of drug molecules through human skin it is necessary to consider the possible routes of penetration and the reservoir function of the skin.

2.2.1 Routes of skin penetration

The skin is an effective barrier to the penetration of the huge variety of physical and chemical toxic agents found in the environment. This barrier function of the normal skin becomes ineffective, however, in the case of certain drugs because their physical, and to some extent chemical, properties allow them to penetrate through the skin into the systemic circulation (Idson 1968 ; Katz and Poulsen 1971).

Katz and Poulsen (1971) have discussed the relative importance of five possible routes of skin penetration. Idson (1971a, 1975) and Barry (1983) reported that there are three distinct routes of skin penetration for molecules coming into contact with human skin, that is through the hair follicles, the sweat ducts and/or through the unbroken stratum corneum between these appendages. Transport through the follicles and sweat ducts forms the "transfollicular" route while penetration through the stratum corneum is referred to as the "transepidermal" route. The combination of the "transfollicular" and the "intercellular"

routes can also be referred to as "shunts" (Figures 1.1 and 2.1).

The process of percutaneous absorption consists mainly of an initial distribution or partitioning of the applied drug molecules at the skin surface and subsequent diffusion through different layers of the skin as shown in Figures 1.2 and 2.1. A circuit diagram (Figure 2.2) can be used to explain the overall phenomenon of percutaneous absorption by recognizing that both resistance in series and diffusional currents in parallel are additive just as they are in electrical circuits. The "transepidermal" and "transfollicular" routes are parallel and electrical current (or a drug) will flow through the pathway of least resistance, with the overall driving force being the concentration differential across the skin (AC). This will be quantitatively equal to the concentration of the drug in the vehicle (C_v) when sink conditions apply. Whether a "transepidermal" or "transfollicular" route is followed will depend upon the relative affinity of these two routes for a particular drug, the fractional surface areas of each of the routes and the ease of diffusion through the respective phases. Regardless of which pathway is followed, the drug must partition into and diffuse through the viable epidermis and dermis to reach the systemic circulation (Witten et al 1951, 1953, 1956; Malkinson 1964; Vickers 1966; Flynn 1979).

The magnitude of each of the diffusion coefficient and lag time (section 2.5, equation 2.6) might indicate the importance of the route of percutaneous penetration. In the case of steroids the small diffusion coefficients and the large lag times (equation

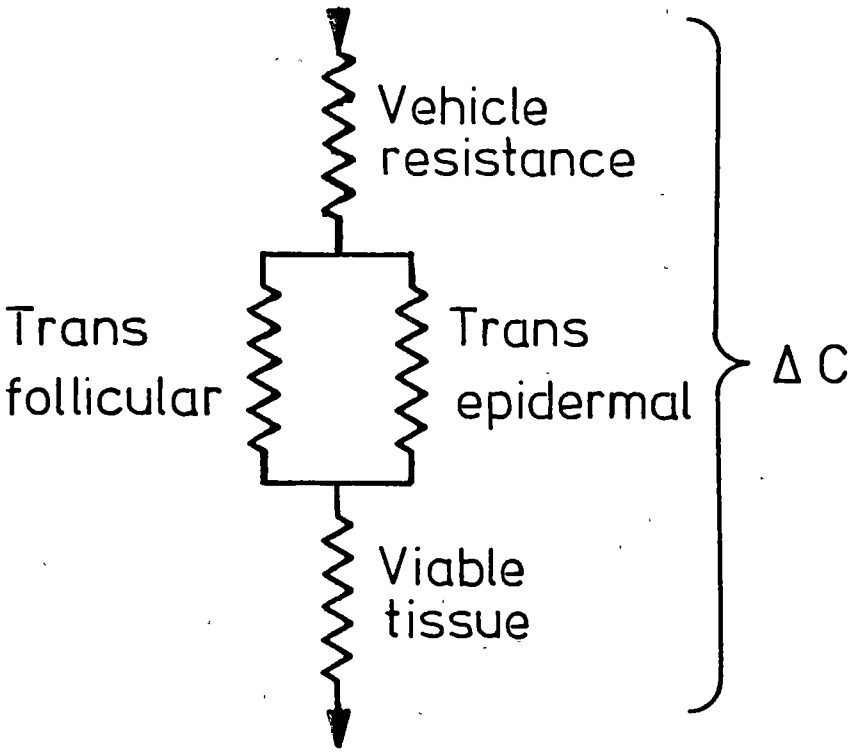


Figure 2.2 Circuit diagram to explain the phenomenon of routes of skin penetration. The fractional area of "transfollicular" and "transepidermal" routes is approximately 0.001 and 1.0 respectively (from: Flynn 1979).

2.6) indicate that the steroids are more likely to penetrate through the "shunts" prior to establishment of the steady state of penetration as indicated in Figure 2.3 (Scheuplein et al 1969; Blank and Scheuplein 1969).

The stratum corneum, is described as a dense homogenous membrane into which non-electrolytes with small molecular weight dissolve with chemical interaction; this permits diffusion to occur at a very slow rate through the "intracellular" route. On penetrating the stratum corneum the rate of diffusion of these substances through the viable epidermis and dermis increases rapidly as shown by the relative diffusion rate coefficients of octanol for stratum corneum (1×10^{-9} cm^2 sec^{-1}), viable epidermis (5×10^{-7} cm^2 sec^{-1}) and for dermis (5×10^{-7} cm^2 sec^{-1}) using *in vitro* techniques (Scheuplein 1966; Micheals et al 1975; Higuchi 1977). Individual ions and large polar molecules can bond with the hydrated keratin of the stratum corneum, which impedes their movement through the "intracellular" route resulting in easier penetration via the "shunts". The *in vitro* and *in vivo* diffusion data for steroids clearly indicates that the percutaneous penetration results mainly through "shunts" (Katz and Poulsen 1971; Barry 1983); this hypothesis was investigated during the present work (Chapter 1).

During the transient stage (that is prior to the establishment of the steady state) significant amounts of a substance may diffuse rapidly through the "shunts". At steady state the stratum corneum accumulates water at the outer surface of the protein filaments. Polar molecules appear to pass through this immobilized water

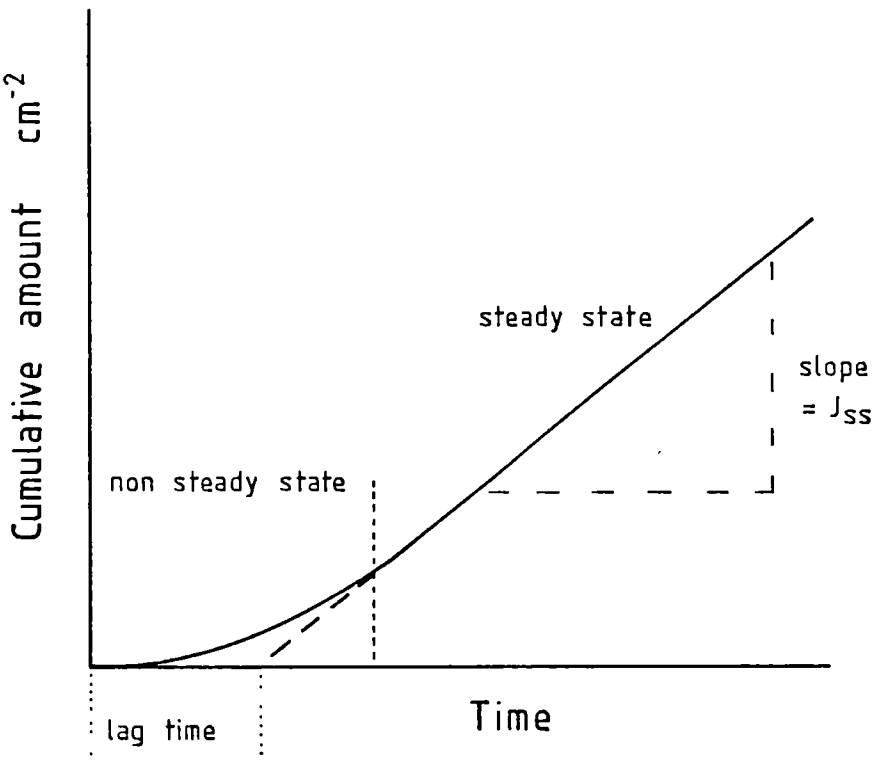


Figure 2.3 A typical permeation profile of drug diffusion through human stratum corneum. J_{ss} is the steady state flux in amount/area/time.

while non-polar molecules dissolve in and diffuse through the non-aqueous lipid matrix between the protein filaments of the stratum corneum. The activation energy for diffusion of polar molecules is generally higher than that of non-polar molecules (Scheuplein 1966, 1967; Blank et al 1967). This theory is contrary to that proposed by Roberts et al (1978) for the permeation of aqueous phenolic compounds through excised human skin; it was concluded by these workers that for more polar solutes the main resistance to the penetration is the lipid barrier in the stratum corneum. In the case of phenols the diffusion through the stratum corneum appears to depend upon the breaking of hydrogen bonds during permeation of phenols. The relative reduction in the rate of penetration of less polar phenols was attributed to the lipoidal nature of the stratum corneum and also to the significant contribution of aqueous boundary layers to overall diffusional resistance. This suggests that with decreasing polarity of the substance the stratum corneum/solute partition coefficient rises until the permeation process becomes diffusion layer controlled.

Higuchi (1977) suggested that the partition coefficients of most polar acidic compounds e.g. phenols and carboxylic acids, and weakly basic substances e.g. amines and sulphoxides appear to be highly compatible with cornified tissue (stratum corneum) and are therefore able to penetrate the barrier with relative ease.

Barry (1983) has summarised the possible routes of penetration for different classes of topical drugs. It has been postulated that for electrolytes and large molecules with low diffusion

coefficients, e.g. polar steroids, antibiotics and possibly ionisable compounds the transport through "shunts" may be significant. However, specific reports by different workers (Scheuplein 1967; Schaefer et al 1982; Barry 1983) indicate that it will be very difficult to determine the relative importance of each route of percutaneous penetration for a particular drug as in situ a substance absorbed through the hair follicles may diffuse in the dermis and may reappear again due to back diffusion, in the subepidermal layers.

2.2.2 Epidermal reservoir for drugs

The possibility of the existence of a reservoir for topically applied drugs within the epidermis first became apparent from the work on the percutaneous absorption of salicylic acid by Guillot (1954) and was later confirmed by Malkinson and Ferguson (1955).

The epidermal reservoir will exist when the concentration of a drug in the upper and intermediary layers of the stratum corneum is substantially higher than that in the viable epidermis and the dermis (Schaefer et al 1982). The stratum corneum is considered to be the main reservoir due to its morphological structure and the resulting barrier function (Schaefer et al 1982).

Vickers (1963) carried out a detailed in vivo investigation on the reservoir or depot properties of the skin. Small quantities of fluorinated steroids in ninety-five percent alcohol were applied to the surface of the skin and the area was occluded with a plastic film. Vasoconstriction or blanching appeared when the film was removed after 16 hours (Mckenzie and Stoughton 1962).

The skin was then thoroughly washed and the subject was allowed to carry out normal activities; the vasoconstriction faded in 10-16 hours. If the areas were re-occluded with plastic film 12-14 days after the first application of steroid, vasoconstriction reappeared indicating that a reservoir for steroids existed in the skin.

Wickrema-sinha et al (1978) applied diflorasone diacetate cream to the skin of human volunteers and twenty four hours later almost 38% of the applied dose had penetrated below the stratum corneum and could not be wiped off. It was concluded that more than 36% of the drug accumulated in the stratum corneum as only 1% of the applied dose was recovered in the urine and the feces. Small quantities of the steroid were recovered from the skin surface for about three weeks after the initial topical application.

In vitro and in vivo tests have indicated that the reservoir is located in the deeper portions of the stratum corneum. The amount of material that can be retained within the reservoir will vary; in the case of steroids the amount deposited in the stratum corneum could influence epidermal cell division. For some drugs the significance of a reservoir, from the practical point of view, is probably more preventative than truly therapeutic. There is however clear experimental evidence (in vivo and in vitro) that hexachlorophane may be an exception as it can be deposited in the stratum corneum in sufficient amounts to be therapeutically active as a bactericide (Munro 1969; Vickers 1972, 1980).

The manipulation of physical and chemical factors to enhance the rate of penetration of different drugs may result in the quantity of the drug in the stratum corneum being increased. Increase in temperature and humidity will increase the rate of percutaneous penetration (sections 2.3.1.1 and 2.3.1.2) which in turn will result in a larger amount of the drug being retained in the skin. Similarly, organic solvents such as dimethylsulphoxide, dimethylacetamide and dimethylformamide which increase the rate of skin penetration of various compounds (section 2.3.1.4) will establish a superior reservoir in the stratum corneum in comparison to other organic solvents such as ethyl alcohol (Vickers 1963, 1972; Fritsch and Stoughton 1963; Stoughton and Fritsch 1964; Stoughton 1965; Kidd 1975).

The barrier and reservoir function of the stratum corneum only allows moderate amounts of a drug to penetrate the skin either for local or systemic effect. Provided that the stratum corneum is not damaged or diseased, the largest fraction of applied drug remains on the skin surface even if an optimal vehicle is employed. Only 0.5%-25% of a drug can penetrate into the viable epidermis for local therapeutic effect. It is wrong however, to conclude that 75% of the topically applied drug is useless. On the contrary, this surplus is a prerequisite for the maintainance of a constant dosage over longer period of time and represents a reservoir from which the drug can penetrate at uniform rates into viable epidermis (Schaefer et al 1982).

2.3 Factors affecting percutaneous absorption

Factors which influence gastrointestinal absorption and those which affect the percutaneous absorption of drugs are similar in nature. In both cases the diffusion rate is mainly dependent upon the physicochemical properties of the administered drug while the formulation factors will only help to enhance the absorption of the drug (Idson 1971a and b, 1975; Barry 1975).

2.3.1 Physicochemical factors

There is general agreement among dermatological investigators that percutaneous absorption occurs by a passive diffusion process with the stratum corneum being the main rate limiting barrier (Idson 1978). It is assumed that percutaneous absorption occurs mainly through the "transepidermal" route; it is recognized however that transport can also occur via the "transfollicular" route. The rate of transport through either route can be enhanced or inhibited by altering various physicochemical factors which are related to the barrier function of the stratum corneum or the physicochemical properties of the administered drug (Wurster 1978).

2.3.1.1 Degree of skin hydration

The stratum corneum becomes hydrated as a result of water diffusing from the underlying epidermal layer or from the perspiration that accumulates after application of an occlusive vehicle or covering on the surface of the skin. The water in the structure of the stratum corneum is either associated with the protein and held in a highly immobile state (bound water) or it

is associated less specifically with the structural components of the stratum corneum and is known as free water (Duzee 1978). The occlusion of skin normally increases its water content from 5% to 50% thus increasing the permeability of most drugs four to five fold. The effect of occlusion on percutaneous absorption has been demonstrated experimentally by measuring the LD50 of dinitrobutyl phenol in rats. The value of the LD50 under occluded conditions was found to be about one fifth of that determined under non-occluded conditions (Idson 1975, 1978).

The degree of hydration of the stratum corneum and its importance in increasing the percutaneous absorption of major topical drugs (Tables 2.2 and 2.3) has been investigated by several workers and discussed in a number of reviews (Idson 1971a,b,1975 and 1978; Katz and Poulsen 1971; Wurster 1978; Schaefer et al 1982; Barry 1983).

2.3.1.2 Skin temperature

Under normal in vivo conditions the skin has a very narrow temperature range. The stratum corneum in temperate climates has a temperature range between 30°-37°C (Barry 1983). If any clinical variation in the penetration of drugs applied to the skin is observed it is mainly due to occlusion (Idson 1978). On the other hand in vitro experiments may be conducted over a much wider range of temperature with an assumption that the stratum corneum does not change on exposure to high temperature (Table 2.4). Duzee (1978) reported that the rheological properties of stratum corneum do not change in the temperature range of 2° to 45°C. Allenby et al (1969a, b) showed however that an abrupt and

Table 2.2 Effect of hydration on percutaneous absorption

Substances	Methods	Results	References
Salicylates	occlusion[h]	increase in percutaneous absorption[*]	Wurster and Kramer [1961]
Hydrocortisone	occlusion[h]	Tenfold increase in urinary excretion rates[*]	Feldman and Maibach [1965]
Nandralone	occlusion[h]	Marginal effect on diffusion parameters[*]	Foreman et al. [1978]
Highly polar non-electrolytes e.g. Water, methanol and ethanol.	Ten hour of immersion in water[a]	No increase in permeability[+]	Behl et al. (1980, 1983)
Hydrophobic alcohols. e.g. butanol and hexanol	Ten hour of immersion in water[a]	Two fold increase in permeability[+]	Behl et al. [1980, 1983]
Mannitol, Ibuprofen and Flurbiprofen	occlusion[h]	Little effect on permeability[+]	Barry [1983]
Water	increase in environmental RH[h] from 0-80%	Little change in trans-sepidermal water loss and permeability of water[+]	Blank et al. (1984)

* in vivo
\+ in vitro

a - animal skin
h - human skin

Table 2.3 Theoretically expected effects of common vehicles on skin hydration and skin permeability - in approximate order of decreasing hydration effect [From Barry 1983].

Vehicle	Examples/ constituents	Effect on skin skin hydration	Effect on skin permeability
Occlusive Dressing	Saran Wrap Melinex Film, unperforated water-proof plaster	Prevents water loss; full hydration	Marked increase
Lipophilic	Paraffins, oils, fats, waxes, fatty acids, alcohols, esters, silicones	Prevents water loss; may produce full hydration	Marked increase
Absorption base	Anhydrous lipid material plus water/oil emulsifiers	Prevents water loss; marked hydration	Marked increase
Emulsifying base	Anhydrous lipid material plus oil/ water emulsifiers	Prevents water loss; marked hydration	Marked increase
Water/oil emulsion	Oily creams	Retards water loss; raised hydration	Increase
Oil/water emulsion	Aqueous creams	May donate water; slight hydration increase	Slight increase?
Humectant	Water-soluble bases, glycerol, glycols	May withdraw water; decreased hydration	Can decrease or act as penetration enhancer
Powder	Clays, organics, inorganics, "shake" lotions	Aid water evaporation; decreased excess hydration	Little effect on stratum corneum

Table 2.4 Effect of temperature on skin permeability.

Substances	Results	References
Aniline	Increase in in vivo[h] penetration from 0.18 mg cm^{-2} hr^{-1} [29.8°C] to 0.72 mg cm^{-2} hr^{-1} [35°C]	Piotrowski [1957]
Aspirin	Increase in in vitro [h] flux by factors of eight [10°C, 88% RH] to fifteen [40°C, 50% RH].	Fritsch and Stoughton [1963]
Polar alcohols e.g. ethanol and propanol	No change in average activation energy [16.5 K cal mol^{-1}][h] over temperature range of 5°C to 50°C [in vitro][h]	Blank and Scheuplein [1964] Blank et al. [1967]
Non-polar alcohols e.g. hexanol and octanol	Reduction in average activation energy from 17 K cal mol^{-1} [<25°C] to 10 K cal mol^{-1} [>25°C].	Blank and Scheuplein [1964], Blank et al. [1967]
N-octylamine	increase in in situ[h] [volar forearm] penetration [1.7°C to 46.1°C]	Cummings [1969]
T-20	Increase in in vitro[a] permeability from 20 ucm min^{-1} [20°C] to 54 ucm min^{-1} [37°C]	Creasey et al. [1978]
Phenols	Increase in in vitro[h] diffusion coefficient with increase in temperature [12.6°C - 34.5°C]	Roberts et al. [1978]
Any substance	The penetration rate of a material through human skin can change [more likely to increase] if the body is exposed to a large temperature change e.g. 30°C to 80°C.	Barry [1983]
	Increase in temperature results in development of erythema; the increase blood flow accelerates clearance of the substance from the skin.	Schaefer et al. [1982]

a - animal skin h - human skin

irreversible structural change can occur in excised stratum corneum when exposed to temperatures above 60°C.

Katz and Poulsen (1971) concluded that the evidence in the literature indicates that there is a direct and real relationship between temperature and skin permeability. The clinical importance of temperature effects in topical therapy is however of minor importance. Even though a solvent with a low boiling point in a topical medication will cool the skin as it evaporates, such an effect is transitory as normal skin temperature is quickly regained. There is a slight increase in temperature on occlusion of the skin due to loss of heat by radiation and by preventing evaporation of sweat. The effect on permeability induced by this increase in temperature is probably small in comparison to the effect due to increased hydration of the stratum corneum consequent upon the presence of the occlusive covering. The general increase in the rate of penetration of a drug caused by occlusion (Tables 2.2,2.3,2.4) is more likely therefore, to be due to the combined effects of temperature and moisture.

2.3.1.3 Significance of drug concentration

The rate of penetration of a drug may depend on the concentration of the drug present in the vehicle. An increase in the amount of a drug in the vehicle, or with application of a drug system of constant concentration to a larger surface area (with or without increased duration of skin contact), will generally produce a significant increase in the rate of penetration of the drug through the skin (Idson 1971). This is not however true for all

compounds as it has been reported that phenols at high concentrations produce caustic effects which impede effective penetration, due to formation of an artificial barrier in the case of animal skin (Macht 1938). Roberts et al (1977a) reported that the penetration of phenolic compounds is concentration dependent and a constant permeability coefficient is observed below a threshold concentration above which the permeability coefficient increases with concentration. This increase in permeability coefficient was attributed to damage to the human epidermis resulting in reduction in its diffusional resistance.

It is only the dissolved drug which is capable of diffusing from the vehicle phase and therefore penetrating the skin. Consequently a poorly soluble drug which is completely dissolved in the vehicle will penetrate faster than an undissolved drug with a high concentration, as for example in a suspension. In the case of compounds which ionise the dissociation constant of the drug and the pH of the vehicle are important factors in determining the effective concentration of the diffusing species, as only the unionised form of the drug may penetrate the skin (Katz and Poulsen 1971; Barry 1983). However, this hypothesis has now been disputed by some workers (Chapter 1 and section 2.3.1.7).

Maibach and Feldman (1969) demonstrated that higher concentrations of hydrocortisone, cortisone, salicylic acid and benzoic acid produced a positive effect on the rate of *in vivo* penetration of these drugs through human skin. It has also been reported by Skog and Wahlberg (1964) that there was a marked

increase in the rate of percutaneous penetration of various compounds in guinea pigs with increasing concentration. This was found to be valid to a certain point beyond which a plateau is reached. This may indicate that the stratum corneum may not be influenced by diffusion gradients and merely acts as an absolute limiting step, which limits the total amount of any substance penetrating through the skin in a unit time (section 2.2.2). In steady state diffusion, the permeability coefficient (Figure 2.3 and section 2.5) is independent of concentration.

Katz and Poulsen (1971) emphasize that it is the effective drug concentration that will control the rate of percutaneous absorption. In the case of an emulsion it is only the concentration of the drug in the external phase in contact with the skin which influences the penetration rate; similarly adsorption to the solid components in a complicated topical vehicle can effectively lower the concentration of the drug. Other factors which may produce a change in effective drug concentration after application will include the loss of volatile component, uptake of water from the skin or atmosphere, phase inversion of an emulsion and the stability of a suspension.

2.3.1.4 The effect of solvents, vehicles and surfactants

It will probably be impossible to find the ideal solvent, that is one which is inert and simultaneously possesses a unique property which will temporarily abolish the barrier function of the stratum corneum and therefore allow topical drugs to elicit systemic effects. Water is commonly used as a solvent because it has the ability to increase the absorption of drugs by increasing

the hydration of the skin (Katz and Poulsen 1971).

There are a number of organic and inorganic solvents which have the ability to damage or alter the nature of the stratum corneum in such a way that its diffusional resistance is reduced, by possible production of holes or artificial shunts, and this allows the active ingredients to penetrate at a faster rate. Such substances (that is those which increase the rate of penetration of topically applied drugs) have been called accelerants, sorption promoters or penetration enhancers (Katz and Poulsen 1971; Grasso and Lansdown 1972). Dimethylsulphoxide (DMSO), N,N-dimethylactamide (DMAC) and N,N-dimethylformamide (DMF) are the organic solvents which have been reported to increase the permeability of a number of drugs by alteration of the barrier resistance of the stratum corneum. These changes in barrier resistance were found to be of short duration with the stratum corneum returning to its normal barrier function within six hours (Baker 1968; Parker and Bailie 1982; Hadgraft 1984). Some of the drugs whose penetration through the human skin has been reported to be increased in the presence of DMSO include scopolamine (Chandrasekaran et al 1977, 1978), salicylic acid and sodium salicylate (Marcus et al 1970; Shen et al 1976), antibiotics (Vickers 1969) and steroids (Stoughton and Fritsch 1964).

In vitro experiments found DMSO, DMAC and DMF to be superior vehicles for increasing the penetration of hydrocortisone and griseofulvin in comparison with other vehicles like ethanol, benzene and a cream base. Studies on the ability of selected solvents to increase the transepidermal water loss showed DMSO to

be most effective with DMAC being less effective than DMF (Munro and Stoughton 1965; Baker 1968; Munro 1969; Astley and Levine 1976). In addition to DMSO, DMAC and DMF other solvents such as benzene, ether and alcohol have been shown to enhance percutaneous absorption of both water soluble and lipid soluble substances. Isopropanol, xylene, urea, methanol, chloroform, propylene glycol and phenol have also been reported to increase percutaneous absorption of a variety of drugs (Grasso and Lansdown 1972). Windheuser et al (1982) have reported that the use of N,N-diethyl-m-tolamide enhances the dermal and transdermal penetration of hydrocortisone in both in vivo and in vitro studies.

Surfactants also appear to be an important contributor in promoting percutaneous absorption (Idson 1971a). One or more surface active agents are usually used in skin preparations as emulsifying or solubilizing agents and also most of the detergents contain a synthetic surface active agent like sodium lauryl sulphate. Stratum corneum is an effective barrier to most surfactants but irritant action observed during the use of certain detergents indicates that ionic surface active agents penetrate the skin, as it is necessary for a substances to penetrate as far as the malphigian layer of the epidermis to exert its irritant effect (Wahlberg 1968b; Idson 1975). Different mechanisms have been suggested for the increase in the percutaneous penetration of a number of substances in the presence of surfactants by Sprott (1965); Scheuplein and Ross (1970); Scheuplein and Blank (1971); Mezei and Ryan (1972); Chowhan and Pritchard (1978); Chowhan et al (1978) and, Dalvi and

Zatz (1982).

2.3.1.5 Skin/drug/vehicle interactions

The interaction between drug/skin, vehicle/skin and drug/vehicle will influence the rate at which the drug penetrates the skin (Katz and Poulsen 1971; Roberts and Anderson 1975; Roberts and Horlock 1978).

When a drug is applied to the skin it produces a pharmacological action to enable it to penetrate through the skin. The degree or duration of this pharmacological action may be influenced by the drug/skin interaction. Due to the complex chemical nature of the skin many substances interact with it to either produce a strong chemical bond or Van-der Waals type of attraction. These bonds could damage the skin or strongly bind the drug to the skin as has been reported by Idson (1967); Roberts et al (1974); Roberts and Anderson (1975) and Dalvi and Zatz (1982).

A drug/skin interaction could influence the hydration state of the skin which in turn will effect the permeability of the skin. Some steroids (hydrocortisone and betamethasone 17-valerate) are metabolized by the human skin resulting in the loss of their anti-inflammatory action. In addition vasoconstrictor effect produced on application of steroids may not only slow their own penetration but might also affect the absorption of other concomitantly applied drugs (Katz and Poulsen 1972; Brookes et al 1982).

The environmental factors to which the human skin is exposed are the temperature, relative humidity (RH) and the rate of movement

of air over the skin surface. Topical administration of any type of dosage form, containing a vehicle, may result in a vehicle/skin interaction by altering one or more of these factors. The mechanism by which the skin permeability may be altered due to its interaction will involve the hydration state of the stratum corneum, temperature of the skin and/or the nature of the vehicle being used (Tables 2.2, 2.3 and 2.4).

A drug/vehicle interaction can result in a slow diffusion of the drug from the vehicle phase onto the skin surface and this will effect the rate of diffusion of the drug across the stratum corneum. It is necessary for a topical dosage form (solution, cream base or suspension) to release the drug onto the skin at a reasonable rate and in adequate amount to enable it to achieve the necessary therapeutic action in the epidermis to cure the skin disorder in the shortest possible time (Katz and Poulsen 1971).

The mathematical relationship (section 2.5), used to describe the rate of release of a drug from a solution or a suspension indicates that the release rate of a drug from a vehicle can be manipulated by changing the drug concentration, varying the diffusion coefficient and increasing the solubility of the drug in the vehicle.

Katz and Poulsen (1971) concluded that because of the presence of a large number of excipients (surfactants, buffers, dyes, thickeners, plasticizers etc) in a topical medication, there are very few topical vehicles that are simple homogeneous systems. Therefore the possibility of a drug/vehicle interaction in a

heterogenous vehicle cannot be overlooked as it might affect the percutaneous absorption of the active ingredient.

2.3.1.6 Solubility and molecular characteristics of the drug

The Meyer-Overton theory of absorption states that substances soluble in lipids pass through the cell membrane because of its lipid content and that water soluble substances penetrate the skin due to the hydration of the protein molecules (Idson 1971a, b and 1975). This theory has been validated by a number of workers and the rates of percutaneous absorption of different drugs, including steroids, have been correlated with their ether/water, octanol/water and benzene/water partition coefficients. It is the aqueous solubility of a drug which determines the concentration presented to the absorption site while the partition coefficient strongly influences the rate at which the drug is absorbed through the skin (Idson 1971a, b and 1975). Blank and Scheuplein (1964), and Scheuplein et al (1969) have reported that in a molecular series of compounds, with a constant diffusion coefficient, the rate at which the drug penetrates the skin (flux) and permeabilty coefficients are directly proportional to the relative solubility of the drug in the skin-that is the stratum corneum/vehicle partition coefficient. This hypothesis might not, however, be true for small molecular substances as Higuchi (1977) has pointed out that the maximal effective thermodynamic activity of a pure drug is more important than its partition coefficient.

Scheuplein et al (1969) confirmed that small molecules penetrate more rapidly than large molecules (e.g. steroids) but within a

narrow range there is little correlation between the size and penetration rate as the diffusion constants through hydrated stratum corneum are similar for many low molecular weight compounds. On the other hand Idson (1975) has suggested that the size and shape of a molecule may also be a significant factor and that an inverse relationship could be found between flux and the molecular weight of a substance.

2.3.1.7 Degree of ionisation

According to the pH-partition hypothesis only the unionised moiety can permeate the lipid membrane in significant amounts (Shore et al 1957). However, it has been suggested that ionised species can also permeate the lipid membrane (Notari 1982).

Ionised species do not have favourable free energies for transfer to the lipid phases, as weak acid and weak bases are dissociated to different degrees depending upon the pH of the solvent and pK_a of the drug. Thus the fraction of the unionised drug in the topical dosage form may determine the effective membrane gradient for ionisable drugs (Katz and Poulsen 1971; Barry 1983). It is recognised however, that individual ions (sodium and potassium) and electrolytes (sodium chloride) can be easily absorbed through the human and animal skin (Tregear 1966a; Schaefer et al 1982).

2.3.1.8 Miscellaneous factors

Other factors such as the particle size of the suspended drug, the viscosity, surface tension and/or volatility of the vehicle(s), and also the polymorphism of certain drugs have also been reported to effect their percutaneous absorption (Idson

1971a; Schaefer et al 1982).

2.3.2 Physiological factors

The major physiological factors influencing percutaneous absorption are skin condition, skin age, regional skin sites, blood flow and variation among species. Practically, some are more significant than others.

An intact stratum corneum is the main barrier; when it is damaged by physical or chemical agents faster penetration of the substances follows. Potts et al (1984) investigated the changes with age in the moisture content of human skin and their results show that aged skin (> 40 years) has a lower water content than does the skin of younger persons. Since it has been shown that percutaneous absorption of topical steroids occurs more readily in children than in adults (Idson 1975), this might be the reason for infant skin being more permeable than adult skin.

Marzulli (1962) pointed out that there is a wide variation in the absorption rate of the same substance from the same skin site in different individuals. The difference is so significant that the rate of penetration for the most permeable region (post auricular skin) in some individuals is comparable to the rate for the least permeable region (sole of the feet) in other individuals. Shaw and Chandraskeran (1978) confirmed that in humans the post auricular skin is relatively more permeable than the thigh skin mainly because of the thickness of the epidermis. Similar results were reported by Roberts et al (1982), who showed that the skin permeabilty for methyl salicylate absorbed <u>in vivo</u> from different

areas of the human body was in the rank order: abdomen > forearm > instep > heel > plantar for all subjects. Feldman and Maibach (1967) have reported that absorption of hydrocortisone occurs from all regions of the human body except the heel, with greater absorption in areas where the follicles are numerous and decreased where the stratum corneum is thickest. Southwell et al (1984) investigated the variation in permeability within and between specimens. Inter-specimen variation, both in vivo and in vitro, was found to be higher than intra-specimen variation.

Kligman (1983) suggested that the thickness of the stratum corneum is unlikely to determine the permeability of a drug(s). In fact the palms of the hand and soles of the feet (section 2.1.1) are far more permeable than the skin of the forearm, as the epidermal water loss through the palms of the hand in vivo (in the absence of sweating) is considerably greater than that from other parts of the body (Scheuplein and Blank 1971; Kligman 1983). It has also been suggested by Kligman (1983) that the absence of erythma or blanching during administration of steroids (section 2.2.2) on to the palms or soles, does not indicate the high impermeability of this region. In fact the thick stratum corneum prevents the attainment of high concentration in the viable epidermis (Figure 1.2); thus the percutaneous absorption may be greater on the palms of the hand but the concentration of the drug in the vicinity of the blood vessels is too low to cause vasodilation or vasoconstriction (Kligman 1983).

In the case of gas permeation an increase in blood flow through the vessels will increase clearance of the gas at a faster rate

although clinically there is no such evidence for topical substances (Idson 1975). It is recognised however, that percutaneous absorption is not only dependent upon a number of factors (section 2.3.1) but the *in vivo* perfusion rate may also increase the percutaneous rate of absorption of any drug (Table 2.4).

The physical characteristics of human and animal skin show wide differences in permeability for various substances but the general order is monkey > dog > cat > horse > rabbit > goat > rat > guinea pig > mouse and forearm skin of man. The human skin is very much less permeable to ions in comparison with that of the rabbit or pig (Tregear 1964; Marzulli et al 1969).

In vitro or *in vivo* studies using animal skin cannot be a substitute for human skin because the absence of sweat glands and/or hairs in certain species would make it difficult to account for the shunt transport which might play an important role in the rate of penetration of certain drugs. In spite of these limitations animal models have to be used to study percutaneous absorption of biologically dangerous substances.

2.4 *In vitro* and *in vivo* methods for studying percutaneous absorption

The main objection to the use of *in vitro* methods is that such a system does not simulate the *in vivo* condition, in which an efficient and variable blood supply removes the penetrating drug at various rates or, in the case of steroids, the occurrence of vasoconstriction which decreases the rate of removal of steroid

from the dermis. In vitro methods may be of limited value but they are an important means of assessing the ability of a vehicle to release the drug, to determine the general routes of penetration and to compare the efficacy of various solvents, vehicles or other added adjuvants on percutaneous absorption from a new dosage form.

Franz (1975) studied the in vitro permeability of twelve organic compounds and compared the results with those of a previous in vivo study carried out on the same compounds by Feldman and Maibach (1970). The quantitative agreement was not perfect but there was an excellent qualitative agreement between the in vivo and in vitro data. The in vitro method which he used adequately distinguished compounds of low permeability from those of high permeability and ranked these in similar order to the results found in vivo. Bronaugh et al (1982) also obtained a good qualitative and quantitative agreement between in vitro and in vivo data for the permeation of benzoic acid, aspirin and urea through rat skin.

The methods used for the in vitro measurement of percutaneous absorption vary considerably in detail but they follow a general pattern. A human or animal skin is trimmed to the required size and is placed so that it divides a permeation cell into two compartments. The rate of permeation of a drug may be measured by following the disappearance of the test substance from the donor compartment or by monitoring its appearance in the receptor compartment or by both methods combined. The amount of the drug retained in the skin can be measured by mass balance after

determining the loss of the drug from the donor and amount in the receptor compartment. The problem with this technique is that the amount of the active ingredient penetrating is so small that it is likely to equal or exceed the accuracy of the analysis (Barr 1962; Stoughton 1964; Katz and Poulsen 1971; Grasso and Lansdown 1972; Schaefer et al 1982).

It is the epidermis or the stratum corneum which is most commonly utilized in in vitro studies. The use of the whole excised human skin including the dermis should be avoided as in vivo the drug is probably removed near the dermo-epidermal junction by the blood vessels. It is abdominal, breast or thigh skin of cadavers removed during surgery or postmortem which is generally utilized in in vitro studies. The methods of Kligman and Christophers (1963) are most commonly used to isolate the epidermis or the stratum corneum from the excised human or animal skin.

Harrison et al (1984) reported that there was no significant difference between the permeation of water through human skin which was recently excised or that which had been frozen at -20°C for up to 466 days. However, Swarbrick et al (1982) have reported that the in vitro permeation of compounds through excised human skin may be affected by several variables including the methods of treating and storing the skin prior to use, as well as the design and operation of the permeation cells. It was reported that the rate of drug permeation through samples of the epidermis that were allowed to dry under controlled humidity conditions at room temperature and rehydrated for one hour before use were found to be similar to those obtained with "new" skin.

Use of frozen skin samples should be avoided as the in vitro rate of permeation was faster than that obtained with "new" skin samples.

The reviews of Nugent and Wood (1980), Schaefer et al (1982) and Barry (1983) describe the different in vivo and in vitro methods used to study percutaneous absorption. The cells used in in vitro studies can be divided into non-steady state and steady state permeation cells. The former uses a flow through receptor phase and the latter uses a stationary receptor phase volume.

It has been shown that with an increase in flow rate there is a significant increase in the in vitro rate of permeation of selected steroids (Crutcher and Maibach 1969), parathion (Anjo et al 1980) and cortisone (Bronaugh and Stewart 1985). On the other hand Pitman and Rosta (1982) demonstrated that there was no significant difference in the rate of penetration of levisamole in a steady state permeation cell at three different stirring rates using sheep skin. It is also interesting to note that Chien and Valia (1984) have recently suggested that horizontal type steady state permeation cells, similar to that used in the present work, are consistently superior to the vertical type cells of Franz (1975), in regard to control of skin temperature and the efficiency of solution mixing.

The choice of method for analysis will depend upon the concentration of the drug recovered in the receptor phase. As the sensitivity of the technique is an important factor, because of the very small concentrations which might permeate the skin, radioactive agents are most commonly used in studying

percutaneous absorption.

2.5 Mathematical models describing skin permeation.

The mathematical relationship describing the permeation of a drug through the stratum corneum is an important consideration in the optimal design of topical dosage forms and drug delivery systems.

Crank (1975) defined "passive" diffusion as a process by which matter is transported from one part of the system to another as a result of random molecular motions. A distinction between permeation and diffusion has been made by Nakano and Patel (1970). Permeation can be regarded as the movement of a drug from a solution on the donor side of the stratum corneum to the receptor side. But before a steady state is established the drug must move through the membrane. This process is diffusion and the rate of diffusion will not necessarily be the same in the period prior to the establishment of the steady state as it will be in the steady state (Figure 2.3).

"Fick's first law of diffusion" states:

$$J = -D \frac{dC}{dx} \qquad \text{Eq. 2.1}$$

Where J (amount/area/time) is the flux of the active ingredient (Q) which diffuses per unit time (t) through a unit area (A) of a membrane. D is the diffusion coefficient (cm^2 sec^{-1}), dc/dx is the concentration gradient with "c" being the concentration and "x" the distance. Flux of the penetrant is equal to the slope of the steady state diffusion curve (Figure 2.3). Therefore Fick's first law of diffusion can be defined as the flux (J) of a

substance which permeates a membrane, with the rate of permeation being directly propotional to the concentration gradient (dc/dx) where "D" is a proportionality constant (Katz and Poulsen 1971).

Fick's first law can also be written in the form:

$$J = KD \frac{dc}{dx} \qquad \text{Eq. 2.2}$$

An expanded form of equation 2.2 has been used by a number of workers (Tregear 1966b; Blank and Scheuplein 1969; Katz and Poulsen 1971; Idson 1971b; Scheuplein 1978b); it can be written in the form:

$$J = k_p \Delta C_s \qquad \text{Eq. 2.3}$$

where k_p (cm time^{-1}) is the permeability constant or coefficient defined by Crank (1975) as being the volume of a gas at standard temperature and pressure passing through 1 cm^2 of the surface of the membrane (or stratum corneum) 1 cm thick per second, when the pressure difference across the membrane is 1 cm of mercury and ΔC_s, which is the difference in the concentration on the two sides of the stratum corneum, will be the drug concentration in the vehicle (C_v) when sink conditions apply. Therefore equation 2.3 transforms into

$$J_{ss} = k_p C_v \qquad \text{Eq. 2.4}$$

or

$$k_p = J_{ss}/C_v \qquad \text{Eq. 2.5}$$

In Figure 2.3 the linear portion of the curve is known as the "steady state" period of diffusion, which is reached when the rate of entry of drug into the stratum corneum is equal to the

rate of exist from the stratum corneum. The non-linear portion of this curve represents the time period required to attain a steady state and is known as the "lag time" (Crank 1975). Lag time (t_L) is defined as the time required for the solute to pass transepidermally through a membrane (skin) of thickness (h) with diffusion constant (D) and is described by the following equation:

$$t_L = h^2/6D \qquad \text{Eq. 2.6}$$

Equation 2.6 is deduced from the solution of Ficks second law of diffusion (equation 2.10; Crank 1975). The use of equation 2.6 is restricted to the case where binding between the drug and the skin does not occur (Katz and Poulsen 1971; Flynn 1979; Scheuplein 1983).

The stratum corneum/vehicle partition coefficient (K_m), which is defined as an index of relative affinity of the solute for the stratum corneum and the vehicle (Hansch 1973), is given by:

$$K_m = C_m/C_v \qquad \text{Eq. 2.7}$$

where C_m is the concentration of the solute in the stratum corneum ($amount_{ss}$/volume of the stratum corneum) and C_v is the concentration of the drug in the vehicle at equilibrium. With the introduction of K_m, an expanded form of equation 2.4 has been reported by Tregear 1966a,b; Blank and Scheuplein 1969; Katz and Poulsen 1971; Idson 1971b; Scheuplein 1978b, 1980, which is in the form of:

$$J_{ss} = DK_mC_v \qquad \text{Eq. 2.8}$$

or

$$k_p = \frac{DKm}{h} = D/h^2 \times K_mh \qquad \text{Eq. 2.9}$$

where h is the thickness of the stratum corneum.

Equation 2.9 only applies to a system which follows Fick's first law of passive diffusion with a flux of a substance being inversely proportional to the area to which the solution is applied, the duration of application and the thickness of the stratum corneum. The diffusion coefficient of the drug, the stratum corneum/vehicle partition coefficient and the concentration of the drug are directly proportional to the rate at which a drug will be absorbed through the skin (equation 2.8).

Katz and Poulsen (1971) pointed out that equation 2.9 is applicable only to the simple diffusional models where steady state diffusion alone (Figure 2.3) is being considered. It has also been pointed out that Fick's first law does not hold for high concentrations of penetrating molecules and that this theory of diffusion does not include the interactions between drug and skin (section 2.3.1.5) which may produce gross changes in the diffusion constant.

Scheuplein (1972, 1978b) suggested that it was not possible to investigate permeation through skin under such restricted conditions therefore Fick's second law (equation 2.10) which takes into account the change in concentration with time caused by diffusion, should be used. A mathematical description of percutaneous absorption can be obtained by solving equation 2.10:

$$\frac{dC}{dt} = D \frac{d^2x}{dx^2} \qquad \text{Eq. 2.10}$$

Various mathematical models (Tables 2.5 and 2.6) have been used to determine different physicochemical parameters involved in

Table 2.5 Diffusion models

Reference	Summary
Higuchi and Higuchi (1960)	A number of theoretical relationships dealing with diffusion through heterogenous barriers were reported. Expressions for kp, D and L have been derived.
Higuchi (1967)	Review of diffusion models
Flynn et al (1974)	Review of diffusion models
Kakemi et al (1975)	Mathematical model was reported for percutaneous absorption of a drug from an ointment. Skin was assummed to be a homogenous barrier. Agreement between calculated and experimental parameters was reported.
Cooper (1976)	A model for estimating in vivo skin permeability coefficient was reported. Expressions were derived for the permeability coefficients in terms of excretion rates and tissue absorption. The usefulness of the model was demonstrated with existing literature data.
Foreman et al (1977)	A method of the measurement of diffusion coefficient for non-occluded skin was reported. This procedure was able to yield values which were reproducible and consistent with alternative methods for the measurement of diffusion behaviour.
Scheuplein [1972;1978b, c,d.]	Basic diffusion equations have been used to develop theoretical concepts involved in assessing various physicochemical parameters of skin permeation.
Albery and Hadgraft (1979)	Equations were derived to describe percutaneous absorption through the epidermal barrier. It was found that theoretically there was no change between drug diffusion through "intracellular" [transcellular] and "intercellular" routes of penetration with time.
Yu et al (1979)	A physical model was reported to describe the delivery of therapeutically active drugs in the skin, with emphasis on the simultaneous transport and bioconversion of the system: prodrug-drug-metabolite. Dermal diffusion coefficient and enzyme rate constants in situ were determined for vidarabine ester.

Table 2.5 [continued]

Reference	Summary
Hadgraft (1979)	Mathematical expressions were derived to describe rates of drug release from controlled release devices. The equations show the effect of slow interfacial transfer between multiphase layers.
Guy and Hadgraft (1980)	Mathematical expressions were derived which relate the degree of percutaneous penetration to the thickness of the applied base. It reports on the relative importance of simple physicochemical parameters and the way in which optimum dosage regimens may be achieved.
Guy and Hadgraft (1983)	Theoretical expressions are derived to predict the amount of a drug reaching the dermal capillaries as a function of simple physicochemical parameters. The relative importance of different process of percutaneous absorption [Figure 1.2] was illustrated.
Albery et al (1983)	A model was presented to describe the transport of esters of nicotinic acid in the dermis. Reasonable values of constants were found to describe the transport and uptake in the dermis.
Ando et al (1984)	A model based on the interaction of solute with stratum corneum was reported. Two types of interactions were proposed ion-dipole and lipid-lipid. A good correlation was found between the calculated and predicted fluxes. The authors suggest that their model provides a means for the prediction of percutaneous absorption solely on the basis of the physicochemical properties of the diffusates.

Table 2.6 Compartmental Models

Reference	Summary
Riegelman (1974)	A review which discusses the pharmacokinetic factors affecting epidermal penetration and percutaneous absorption of a number of drugs. A comparison of urinary excretion of drugs after oral and topical administration was discussed.
Barnett and Licko (1977)	Models for the transfer of solute across epithelial tissue (e.g. skin) was reported. It was concluded that (a) the tissue is a heterogenous barrier (b) transport was not limited by unstirred layers either at the tissue surface or within the tissue itself and (c) parallel transport pathways do exist.
Chandrasekaran et al (1978)	A model for estimating and optimizing the temporal pattern of scopolamine delivery from a transdermal therapeutic system through human skin in vivo has been reported. Experimentally measured scopolamine delivery in vivo conformed to this model.
Wallace and Barnett (1978)	Data for in vitro permeation of methotrexate through hairless mouse skin from vehicles varying in pH 3.5 to 6.5 were computer fitted to estimate model parameters. The penetration through shunt pathway increased as vehicle pH and ionization was increased.
Guy et al (1982)	A model to describe the amount of drug excreted as a function of time following topical application was reported. This model may possibly prove of general applicability for understanding fate of a variety of drugs after topical administration.
Guy et al (1983)	A model to assess the effects of repeated topical dosing was reported. The four first order rate constants in the model were able to describe the excretion profile of two substances [hydrocortisone and malathion] which have different physicochemical properties.
Guy and Hadgraft (1984a)	A model which allows calculation of drug disposition in skin, plasma and urine was reported.
Guy and Hadgraft (1984b)	A model with four first order rate constant assigned to predict penetration, permeation, resorption [Figure 1.2] and reservoir effects within the skin was reported. The results suggest a framework for the prediction of pharmaceutically and clinically relevant information following topical administration of drugs for local or systemic effects.

percutaneous absorption. Diffusion models (Table 2.5) are based on equations developed from Fick's second law of diffusion (equation 2.10) and compartmental models (Table 2.6) are developed from compartmental analysis.

The equations used in most of the mathematical models are complex and have no simple solutions (Hadgraft 1983). After topical application of a drug, the short time of contact with the skin prevents the attainment of steady state conditions (Figure 2.3) which means that the solution of Fick's second law of diffusion (equation 2.10) in the stratum corneum, viable epidermis and dermis is required with appropriate boundary conditions. These equations will be further complicated if the kinetics of metabolism is included (Scheuplein 1972, 1978b; Hadgraft 1983). Therefore the solution of most of the models shown in Table 2.5 are based upon computer simulations and appropriate approximations to predict various physicochemical parameters (J_{ss}, D, k_p, K_m and L) involved in percutaneous absorption.

Compartmental models (Table 2.6) may also be used to predict levels in the skin, blood and urine provided a relatively simple set of equations can be developed. The rate constants employed can then be ascribed to some of the basic physicochemical parameters (k_p, D, K_m) for both routes of skin penetration (Hadgraft 1983).

An important aspect in percutaneous absorption research is to develop a mathematical model which can describe percutaneous absorption solely on the basis of the physicochemical properties of the solute; both a diffusion model (Ando et al 1984) and

compartment model (Guy and Hadgraft 1984b) have been proposed recently as being able to do this. The data presented by both authors support their hypothesis; however substantial additional application of these models to *in vivo* and *in vitro* data of other workers is required before a realistic evaluation can be made.

CHAPTER 3

EXPERIMENTAL METHODS

3.1 Materials

3.1.1 Chemicals

Phenol, Chlorocresol and 1-Octanol were obtained from Ajax Chemical Ltd, Australia. Hydrocortisone, Triamcinolone, Prednisolone, Testosterone, Corticosterone, Triamcinolone acetonide and Methotrexate (amethopterin) were obtained from Sigma Chemical Company, USA. Betamethasone 17-valerate (Glaxo Australia Pty Ltd), Lignocaine hydrochloride (Astra Pharmaceuticals, Australia), Chlorpromazine hydrochloride, (May and Baker Australia Pty Ltd) and Chlorpheniramine maleate (Glaxo Australia Pty Ltd) were gifts from the pharmaceutical companies. Ephedrine hydrochloride BP, Aspirin BP and Salicylic acid BP were purchased from Evans Medical Ltd, England. Pilocarpine hydrochloride was purchased from Macfarlane Smith Ltd, Scotland. Trypsin (salt free, crystalline and lypholized), Ethyl carbamate (urethane) and water for injection were supplied by Boehringer Mannheim, West Germany, BDH Chemicals Ltd, England and Farmer Hill Pty, Australia respectively. Stable plasma protein solution (SPPS: 5g plasma proteins in 100ml buffered solution at pH 7.0) was a gift from the Commonwealth Serum Laboratories, Australia. All other reagents used were of analytical grade. Deionised glass distilled water was used throughout this work.

Hydrocortisone (1,2,6,7-^{3}H(N); 80 ci mmole^{-1}), corticosterone (1,2,6,7-^{3}H(N); 105 ci mmole^{-1}), triamcinolone acetonide (6,7-^{3}H(N); 37 ci mmole^{-1}), and rubidium chloride (Rubidium-86)

were purchased from New England Nuclear, USA. Prednisolone (2,4,6,7-^{3}H; 57 mci mmole^{-1}), methotrexate-sodium salt (3',5',7-^{3}H; 250 mci mmole^{-1}) and sodium chloride (Chlorine-36) were purchased from Amersham International plc, England. Tritiated water (2.3 x 10^{6} dpm g^{-1}) was obtained from Packard Instrument Company, USA. Biofluor (New England Nuclear) was used as liquid scintillation cocktail throughout this work.

Silastic 501-1 (Dow Corning Corporation, USA) was used as an inert membrane (thickness: 0.018 cm), in this work. Apiezon-AP 100 (Apiezon products Ltd, UK) was used as silicone lubricant to prevent leakage from the permeation cells. A medical adhesive (Hollister Inc, USA) was used in in vivo dermal kinetic studies.

3.1.2 Excised human skin

Samples of human skin, including the subcutaneous fat, approximately 25 cm by 6 cm, were removed from mid abdominal region of caucasian cadavers (male or female) aged 50-78 years, within 48 hours of death and stored at -20°C for 2-3 days before the removal of the stratum corneum and dermis. All procedures had previously been approved by the Human Ethics Committee of the University.

3.1.3 Animal skin

Hooded Wistar rats (male, approximately 300g) were used in dermal kinetic studies. The animals were starved overnight before the start of the experimental procedures. Prior to the removal of the epidermis (section 3.2.2) the animals were either sacrificed (with an overdose of ether) or anaesthetised with an

intraperitoneal injection of urethane (as 25% aqueous solution, 1.5 mg g^{-1}). The duration of anaesthesia was between 5 and 6 hours. At the end of the experiment the anaesthetised animals were sacrificed using ether. All procedures had previously been approved by the Animal Experimentation Committee of the University.

3.1.4 Apparatus

An analytical balance (Mettler H10TW; Switzerland) was used to weigh all the reagents and substances. Micropipettes (Sampler system, Oxford, USA), range 0.01 ml to 5 ml with disposable teflon tips were used to measure and withdraw the samples from either the receptor or donor compartments of the permeation cells.

A thermomix (Haake E12 or Heidolph T50; West Germany) was used to maintain temperature in the water bath during permeation experiments and in the isolation of epidermis from full thickness human skin.

A light microscope (SKF-S3, Shimadzu Kalnew, Japan) was used for the examination of the stratum corneum after its separation from the epidermis.

A Metrohm Herisau E520 (Switzerland) pH meter was used for all pH measurements. A centrifuge (GS-100, Clements Pty Ltd, Australia) was also used during this work.

A high pressure liquid chromotographic system (Waters Associates) containing of either a variable wavelength detector (model-450)

or a fixed wavelenght detector (model-441) was used with appropriate columns for all HPLC analyses.

The spectrophotometric determinations were carried out using either a Beckman DB-G (UK) or a Pye-unicam SP8-100 (UK) recording spectrophotometer.

A liquid scintillation counter (Rackbeta II, LKB, Wallac, Finland) and Gamma counter (supplied by Department of Physics, University of Tasmania) was used for radioactive counting.

The peristalic pumps used for perfusion through the permeation cells were Valley Lab (Infutrol, USA), LKB 1200 (Varioperpex, Sweden) and Masterflex (Coleparmer instrument company, USA).

3.2 Method of isolating dermis and the stratum corneum

3.2.1 Human dermis and stratum corneum

The subcutaneous fat was carefully trimmed and the method of Kligman and Christophers (1963) was adapted as follows to remove the epidermis and the stratum corneum. The whole skin was immersed in water at 60 $\pm$ 0.5^{o}C for three minutes. The skin was removed from the water, blotted dry and pinned, dermal side down, to a wooden board. Forceps were used to tease off the intact epidermis from the dermis. The isolated dermis was used for in vitro dermal kinetic studies.

To isolate the stratum corneum the epidermis was floated, dermal side down, in 0.1% trypsin solution in 0.05M trizma buffer at pH 7.90 for 50 minutes. Cotton buds were used to slowly rub off the viable epidermis. The transparent sheet of stratum corneum

obtained was washed several times with water, dried overnight at room temperature and stored at -20°C prior to use. The weight of the stratum corneum (4.5 cm^2) was 72 ± 0.016 mg (n = 4).

3.2.2 Rat dermis

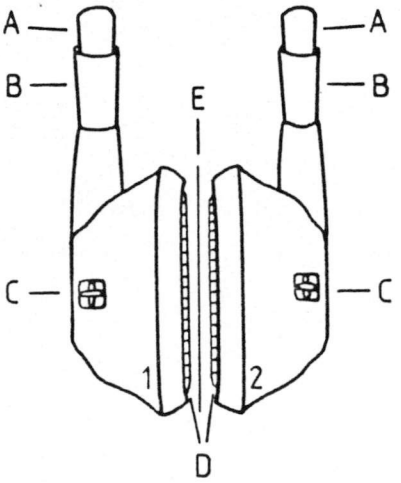
The hair in the abdominal region of dead or anaesthetised rats was shaved with animal clippers and a depilatory agent (Nair; Carter and Wallace Pty. Ltd) was applied to the region for ten minutes to remove the residual hair. The method of Frosch and Kligman (1977) was used to remove the epidermis. Aqueous ammonium hydroxide solution (1:1) was applied to the shaved area (5 cm^2) using small teflon cells to hold the aqueous solution on the skin. The appearance of a blister around the area exposed to the ammonium hydroxide solution enabled the removal of the epidermis. The minimum blistering time with 1:1 ammonium hydoxide solution was found to vary between 50 and 90 minutes for the abdominal skin of the rats.

3.3 In vitro permeation apparatus

3.3.1 Steady state permeation cells

A diagrammatic representation of the glass cell used is shown in Figure 3.1. The clamped glass cells were immersed in a water bath at 25 ± 0.5°C and a constant stirring rate of 40 ± 3 revolutions per minute was maintained in both the compartments using a synchronized motor and external magnets. The maximum capacity of each of the donor and receptor compartments was 3.5 ml (Figure 3.1). The surface area of stratum corneum, dermis or inert membrane exposed to the solution in the cell was 4.5 cm^2.

(a)



(b)

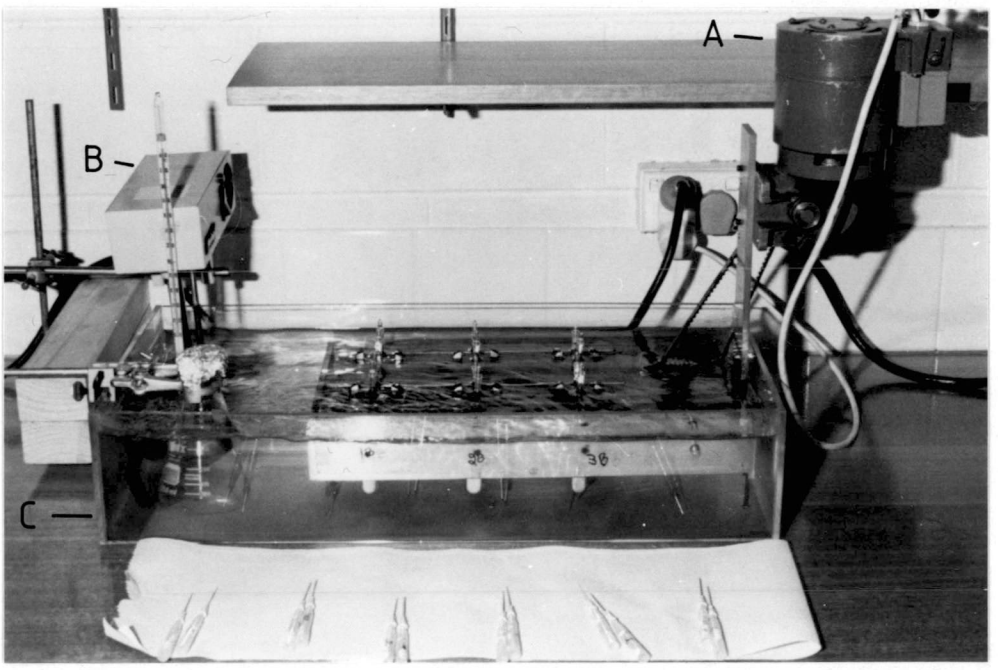


Figure 3.1 (a) Diagram of the apparatus used in permeation experiments. A - glass stoppers, B - sampling ports, C - magnetic fleas, D - wire mesh, E - stratum corneum. A spring loaded clamp is used to hold the donor (1) and receptor (2) compartments together.
(b) Photograph of the apparatus using the above cells. A - synchronized motor, B - thermomix, C - water bath.

3.3.2 Non-steady state permeation cells

Representation of the non steady state glass cells used is shown in Figure 3.2. Control of temperature and stirring was as described in section 3.3.1 for steady state cells except that the stirring of the solution in the donor compartment was carried out using a paddle stirrer attached to a glass rod, which was connected to an external motor. The volume in both donor and receptor compartment was 3.5-4 ml, although the capacity of the donor compartment was approximately 10 ml. Peristalic pumps were used to obtain perfusion rates of 10, 20, 30 and 40 ml hr^{-1} in the receptor compartment. Effluent was collected in a conical flask (Figure 3.2b).

3.3.3 Glassware

All glassware, including the components of the permeation cells, was washed with a laboratory detergent (Pyroneg; Diversey Pty Ltd, Australasia), rinsed thoroughly with tap water and rinsed three times with distilled water before being dried in an oven. Items of glassware and permeation cells previously contaminated by oils or radioactive substance were pre-washed with acetone and chromic acid. Precautions were taken to avoid any cross contamination with radioactive substances.

3.4 Permeation experiments

The stratum corneum (section 3.2.1) was allowed to thaw overnight at room temperature and rehydrated by immersion in water for one hour (Swarbrick et al 1982; section 2.4) prior to being placed in

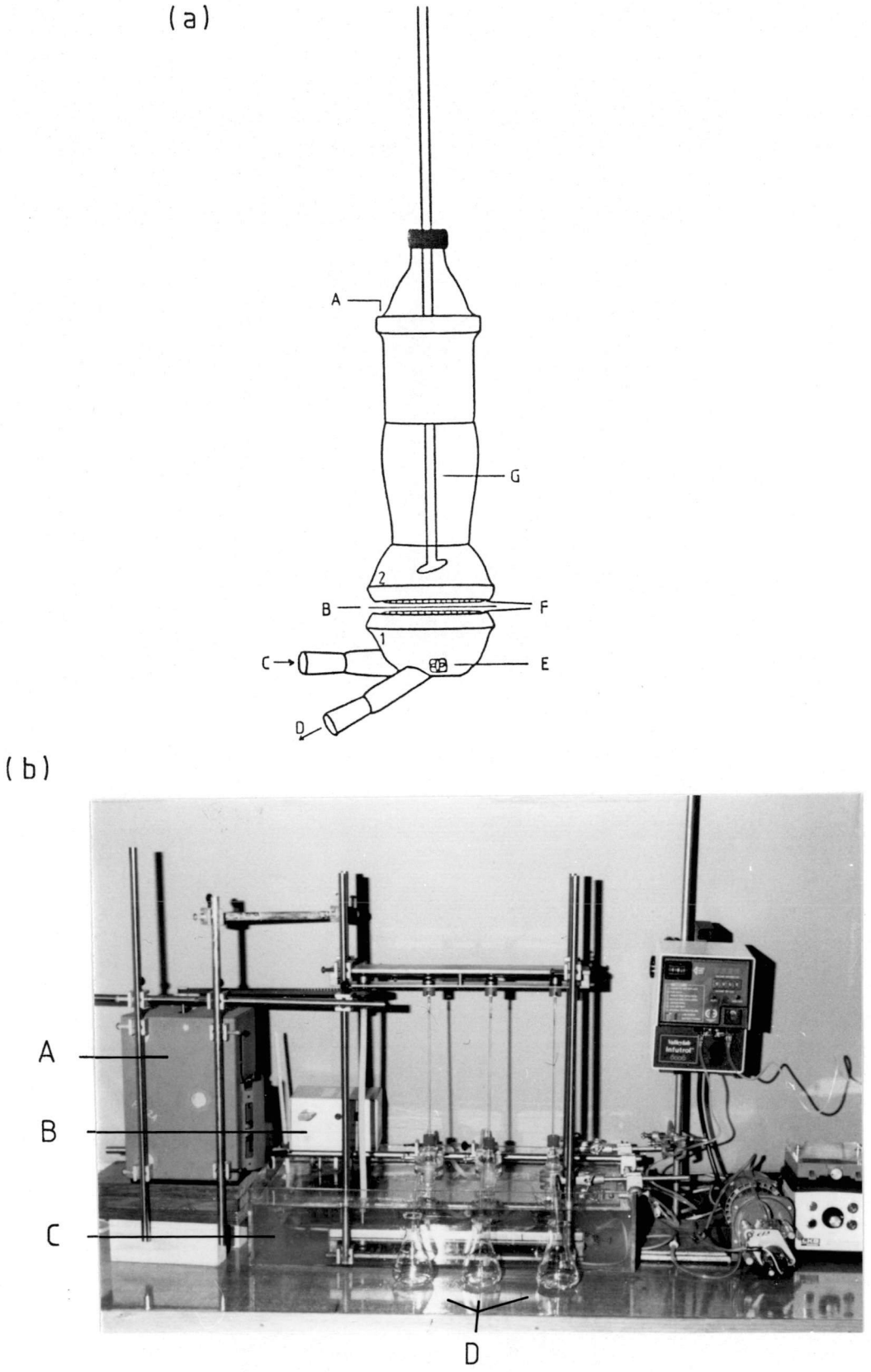


Figure 3.2 (a) Diagram of the non-steady state permeation cell. A - sampling port, B - stratum corneum, C - receptor solution inlet, D - receptor solution outlet, E - magnetic flea, F - wire mesh, G - glass stirrer. A spring loaded clamp was used to hold receptor (1) and donor compartments (2) together. (b) Photograph of the apparatus using the above cells and peristalic pumps. A - synchronized motor, B - thermomix, C - water bath, D - beakers.

the permeation cell with the dermal side towards the receptor compartment. The stratum corneum was supported in this position by a wire mesh (Figures 3.1 and 3.2). A thin film of silicone lubricant was spread on the lapped glass surface of the cells to provide a water tight glass to membrane seal.

The donor compartment contained a solution of a drug (with or without radioactive component) to give final concentrations as shown in Table 3.1. Buffer systems shown in Table 3.2 were used as solvents for weak electrolytes during epidermal permeation studies. The dermal kinetic studies of methotrexate and steroids (pH 7.2) were carried out using buffer-e (Table 3.2). Isotonic sodium chloride containing 0.25% chlorbutol was used as the receptor fluid for all permeation experiments. Stable plasma protein solution (SPPS) containing 0.25% chlorbutol was also used as receptor fluid in steady state permeation experiments. Samples were withdrawn at regular intervals from the receptor side and occasionally from the donor side.

Aliquots of 0.5 ml or 1 ml (Figure 3.1) were withdrawn from the receptor compartments of the cells. Receptor volume was replaced by addition of an equal volume of the original receptor fluid immediately after the sample was removed. In the case of the non-steady state cells larger volumes of perfused receptor fluid were available for analysis. Occasionally samples (0.1 ml) were also withdrawn from the donor compartments of both steady state and non-steady state cells.

The duration of permeation experiments was determined by the period of time taken by a solute to reach a steady state (section

Table 3.1 Selected physicochemical data of the substances.

substance	molecular weight	pK_a^*	concentrations[+] used %	wavelength nm
Water[R]	18	--	--	--
Phenol[H]	94	10.0	0.4	270
Salicylic acid[S]	138	3.0,13.4	0.08,0.008	237
Chlorocresol[H]	143	--	0.2	270
Aspirin[S]	180	3.5	0.05,0.005	237
Ephedrine hydrochloride[S]	202	9.6	0.06,0.006	251
Pilocarpine hydrochloride[S]	245	1.6,6.9	0.007	215
Testosterone[S]	288	--	0.002	240
Lignocaine hydrochloride[S]	289	7.9	0.1,0.01	254
Corticosterone[R]	347	--	0.0024	--
Chlorpromazine hydrochloride[S]	355	9.3	0.004,0.0004	254
Prednisolone[R]	361	--	0.0035	--
Hydrocortisone[R]	363	--	0.003	--
Chlorpheniramine maleate[S]	391	9.2	0.06,0.006	262
Triamcinolone[S]	394	--	0.0024	239

Table 3.1 [continued]

substance	molecular weight	pK_a^*	concentrations+ used %	wavelength nm
Triamcinolone acetonide[R]	435	--	0.0024	--
Methotrexate[R,H,S]	454	4.3,5.5	0.03,0.003	313
Betamethasone 17-valerate[S]	477	--	0.001	241

H - HPLC; R - LSC; and S - spectrophotometer

+ Aqueous solubility range given in British Pharmacopoeia (1980), Martindale (1982), Merck Index (1976) and by Valvani and Yalkowsky (1980) was used.

* From Albert and Serjeant (1984), and Martindale (1982).

Table 3.2 Type of buffer systems.

Buffer	Buffer constituents	Name	Source
a	$C_6H_6Na_2O_7 \cdot 1\frac{1}{2}H_2O/HCl$	Sorensen's buffer	Documenta Geigy (1970)
b	Na_2CH_3COO/CH_3COOH	Citrate buffer	APF (1983)
c	NaH_2PO_4/Na_2HPO_4	Phosphate buffer	APF (1983)
d	KH_2PO_4/Na_2HPO_4	Sorensen's phosphate buffer	APF (1983)
e	$Na_2HPO_4 \cdot 12H_2O/C_6H_8O_7 \cdot H_2O$	McIlvaine buffer	Pharmaceutical handbook (1980)
f	Glycine/NaOH	Sorensen's buffer	Documenta Geigy (1970)

2.5, Figure 2.3). In the case of steroids the permeation experiments were run over a period of 70-90 hours. For weak electrolytes the permeation experiments were generally run for a maximum period of 6 hours, except for methotrexate and pilocarpine where the permeation run was of 25-50 hours duration.

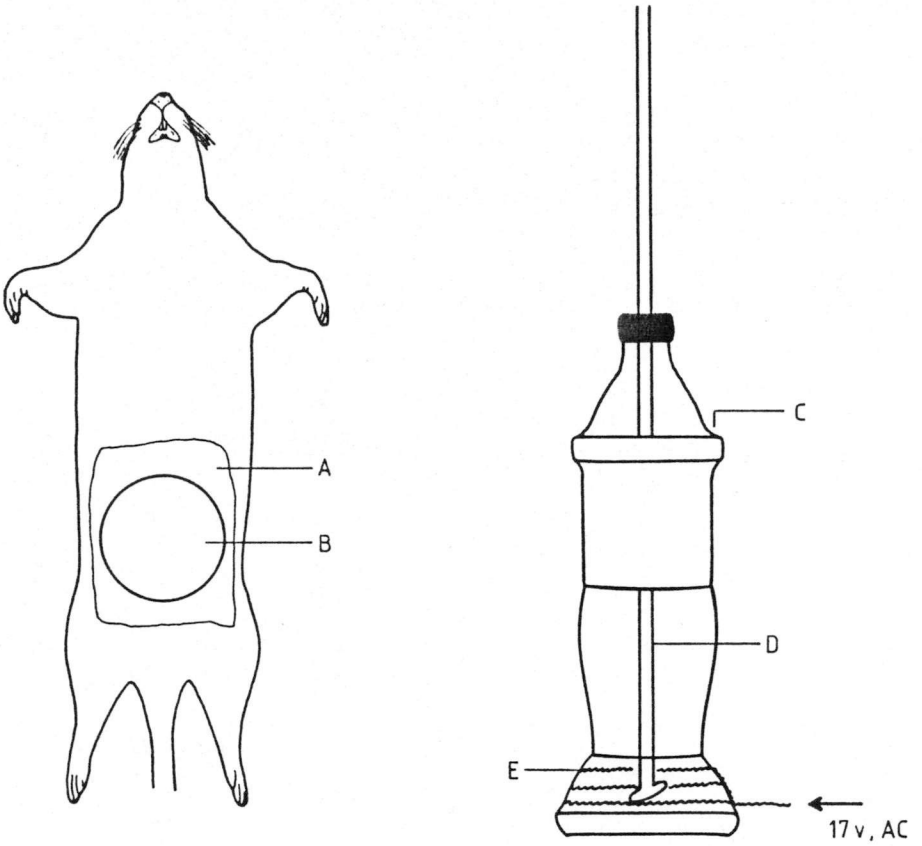
Subsequent to analysis, concentrations and cumulative amount recovered in the receptor compartment for both the steady state and non-steady state studies were calculated. The change in the receptor concentration during sampling (i.e. on addition of the receptor fluid) were taken into account in these calculations. In all studies, the cumulative percentage recovered in the receptor compartment was not allowed to exceed 10% of the initial concentration in the donor compartment. The steady state cells (Figure 3.1) were used for all permeation studies, while the non-steady state cells (Figure 3.2) were only used during the permeation studies of steroids.

3.5 Dermal absorption experiments

The apparatus shown in Figure 3.1 was also used for all in vitro dermal kinetic studies. Human dermis (section 3.2.1) was placed in the permeation cell (Figure 3.1) which was subsequently immersed in a water bath at 25°C or 37°C as described for stratum corneum (section 3.4).

The in vivo dermal kinetic studies on sacrificed and anaesthetised rats were carried out using the apparatus shown in Figure 3.3 and employing the approach similar to that described by Levy and Rowland (1972, 1974). Half permeation cells (Figure

(a)



(b)

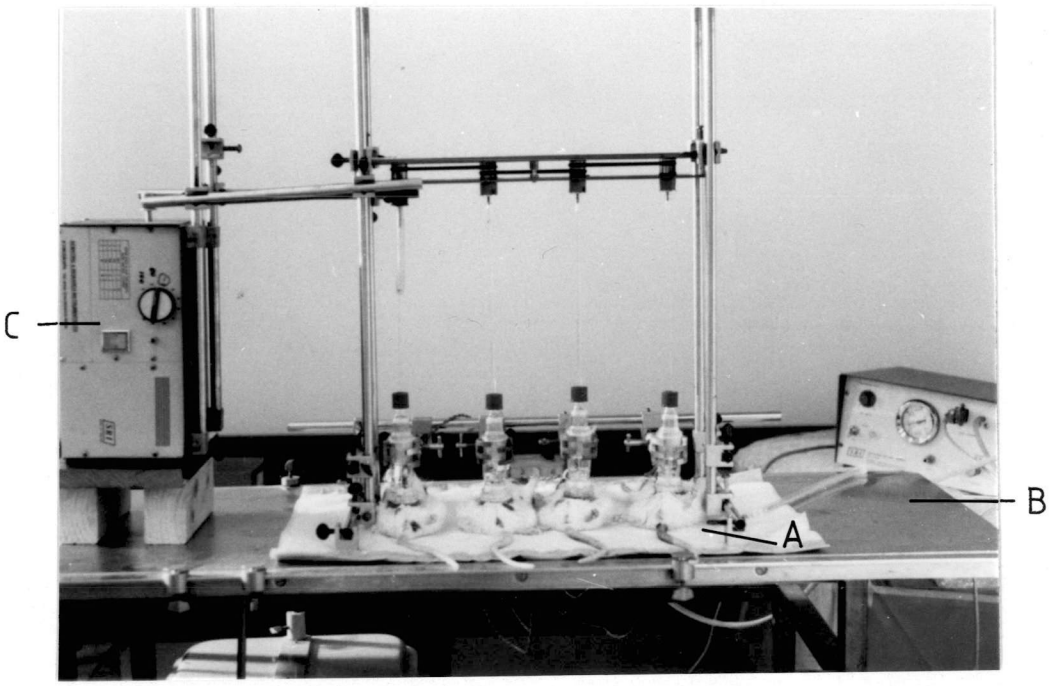


Figure 3.3 (a) Schematic representation of the animal and abdominal dosing area where the glass cell was placed. A - shaved area, B - dosing area (4.5 cm^2), C - sampling port, D - glass stirrer, E - wrapped electric wire to maintain the temperature of the solution at 37°C. Glass cell was placed over the dosing area and held by a retort stand. (b) Photograph of the experiment. A - anaesthetised and dead rats, B - temperature controlled bench, C - synchronized motor.

3.3) were placed on the exposed dermis (section 3.2.2) of the rats using medical adhesive and silicone lubricant. The permeation cell was supported in this position using a retort stand (Figure 3.3).

The solution (pH 7.2; pH of the dermis is between 7.1 and 7.3, from Katz and Poulsen 1971) in the half cell was maintained at 37 $\pm$ 1^{o}C by direct heating of the outer glass surface of the cell with a wrapped electric wire. The solution was stirred at a constant rate of 40 revolutions per minute using an external synchronized motor and a glass rod with paddles (Figure 3.3). Animals were kept warm throughout the whole experimental procedure.

The reduction in concentration in the donor compartment in both in vitro and in vivo dermal kinetic studies was monitored with time using the appropriate analytical method (Table 3.1).

3.6 Iontophoresis experiments

A diagramatic representations of the glass cell with the iontophoresis assembly included is shown in Figure 3.4. The permeation cells were assembled containing the stratum corneum as described in section 3.4. The clamped cells were immersed in a water bath at 25 $\pm$ 0.5^{o}C and a constant stirring rate of 40 revolutions per minute was maintained in both the compartments using a synchronized motor (Figure 3.1b).

The current in the electrodes was generated from a standard 9 volts (direct current) adaptor attached to the mains electric source. The electrodes were placed in the permeation cells,

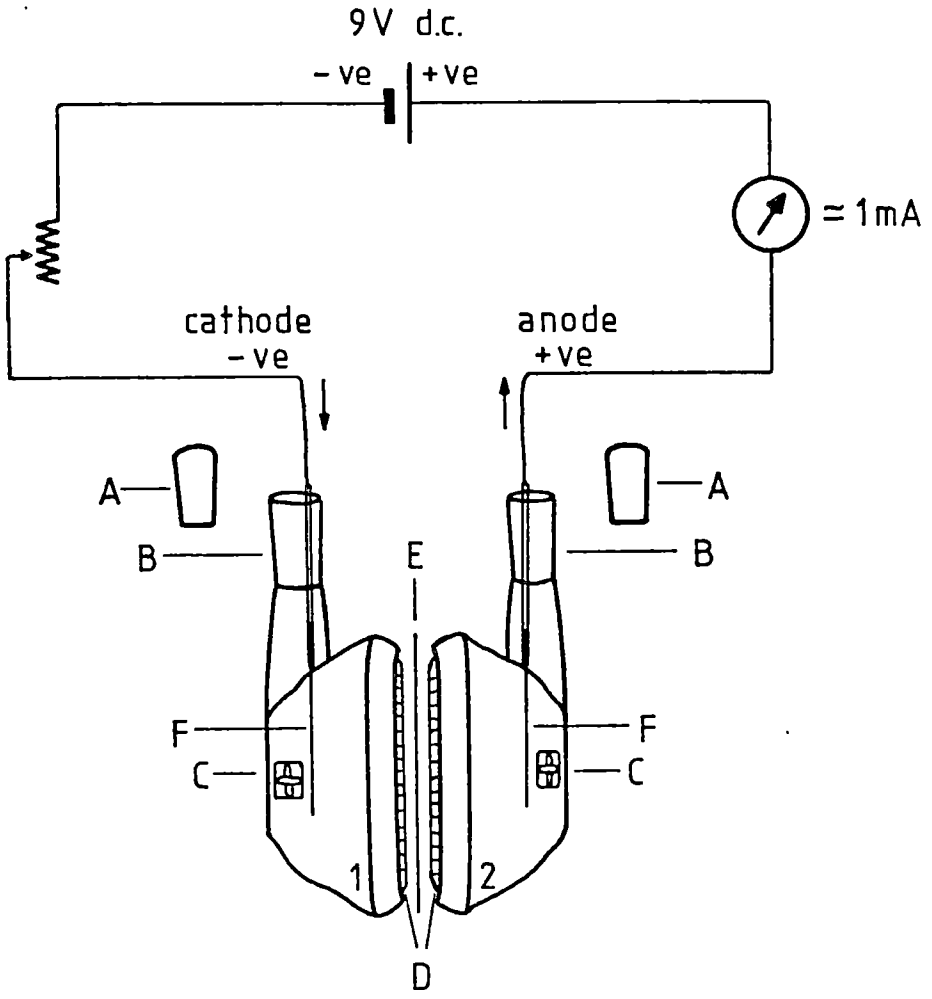


Figure 3.4 Diagram of the apparatus used in permeation experiments and iontophoresis. A - glass stoppers, B - sampling ports, C - magnetic fleas, D - wire mesh, E - stratum corneum, F - platinum electrodes. A spring loaded clamp is used to hold the donor (1) and receptor (2) compartments together.

during the experimental procedure, for 3 minutes. The ionic strength of the buffers, the duration of application of iontophoresis (3 minutes) and magnitude of current (approximately 1 mA) was kept constant for all the permeation experiments using iontophoresis. The anode was placed in the donor compartment for the permeation experiments using basic weak electrolytes while the cathode was placed in the donor compartment during permeation studies using acidic weak electrolytes.

3.7 Methods of analysis

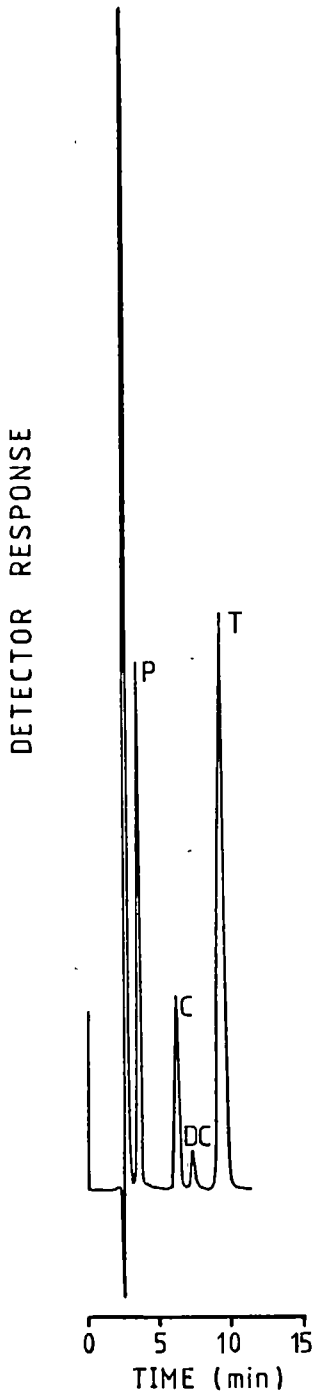
3.7.1 High Performance Liquid Chromatography (HPLC)

Phenol and chlorocresol were analysed using HPLC (Figure 3.5). Concentration was determined by reference to a linear calibration plot of peak height ratio (solute to internal standard) against solute concentration.

Methotrexate was also analysed by HPLC (Figure 3.6) during the *in vitro* and *in vivo* dermal kinetic studies. Concentrations were determined by reference to a previously prepared calibration plot, which was linear.

3.7.2 Thin Layer Chromatography (TLC)

TLC was used to validate the purity of radioactive substances and also to verify the identity of the labelled substances permeating through the human stratum corneum. Thin layer plates (cellulose with luminescer) were used with the appropriate mobile phase, the details of which were sent by the manufacturer of the radioactive substances.



HPLC CONDITIONS

Column: 0·01mm Serva, 250mm × 4·6mm

Mobile phase: 70 % methyl alcohol

Flow rate: ·1 ml/min

Detector: Variable UV, λ = 270nm, 0·1 AUFS with 10 mv recorder.

Ref: Modified method of Gupta (1976)

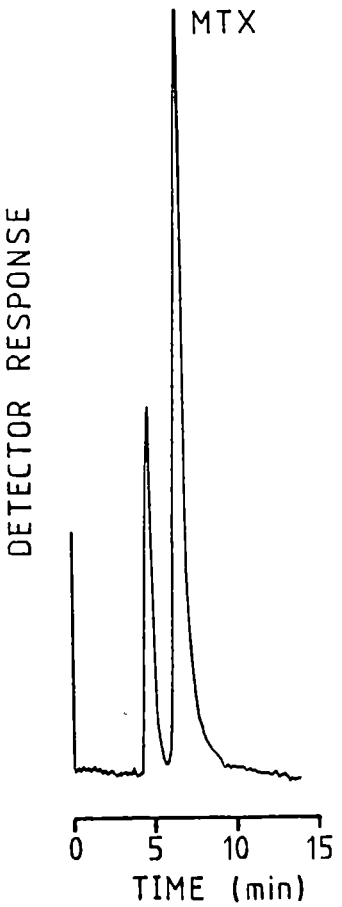
P: Phenol

C: Chlorocresol

DC: Dichlorophenol

T: Thymol (int. std.)

Figure 3.5 HPLC chromatogram of phenol, chlorocresol and dichlorophenol.



HPLC CONDITIONS

Column: 0·01mm Phenyl μBondapak, 300mm × 3·9mm

Mobile phase: 11% acetonitrile in sodium acetate buffer, pH 4·6

Flow-rate: 2 ml/min

Detector: Fixed UV, λ=313nm, 0·01 AUFS with 10mv recorder.

Ref: Canfell and Sadee (1980)

Figure 3.6 HPLC chromatogram of methotrexate.

The British Pharmacopoeia (1980) method was used for the measurement of radioactivity over the length of the plate by dividing the absorbent into strips and removing and counting each portion.

3.7.3 Spectrophotometric analysis

The concentration of a number of solutes was measured spectrophotometrically (Table 3.1). All solutes used obeyed the Beer-Lambert relationship and concentrations were determined by reference to the appropriate calibration plot, each of which was linear.

3.7.4 Radioactive counting

Radioactive counting was carried out using either a liquid scintillation counter (LSC) or a gamma counter. The instruments were previously calibrated using the standard procedure recommended by the manufacturers. Appropriate volume of a solution was added to 10 ml of biofluor for liquid scintillation counting. Counts were measured directly on a gamma counter by exposing the radioactive solution to the head of the counter.

The counting procedure was carried out for thirty minutes in which it was possible to obtain approximately 1000 counts for the majority of the substances. Corrections were made for quenching and counts per minute (CPM) were converted into disintegrations per minute (DPM). Concentration of a solute was calculated using DPM values (Table 3.1).

3.7.5 Data analysis

Individual data representing the cumulative amount of solute recovered in the receptor compartment against time for various pH values (weak electrolytes only) were fitted simultaneously to reduce the standard error between various data points of the same substance, using the non-linear regression programme FUNFIT (Pedersen 1977), appropriate equations and specified weighting on a Burroughs B6800, computer. The goodness of fit and model parameters were compared using the residual analysis techniques and other statistical measures (Boxenbaum et al 1974; Pedersen 1977).

A standard one way analysis of variance and a test of least significant difference was performed using a standard statistical package (Teddybear; a statistical programme written by J.B. Wilson, Botany department, University of Otago, Dunedin, New Zealand) on the same computer. This analysis of variance was performed with factorial arrangement of treatments, where the factors are pH and the steady state flux (with and without iontophoresis). The log transformation was used to equalize variances between the treatments (Snedecor and Cochran 1980).

Linear regression ($y = mx + c$) of the data was also carried out to obtain the slope and intercept for straight lines of best fit.

3.8 Purification of radioactive substances

3.8.1 Methotrexate

As the purity of radioactive methotrexate (^{3}H) decreases on storage, it was purified prior to use. Samples were injected into the HPLC and the fractions of purified methotrexate were collected (Figure 3.7). The aqueous mobile phase was subsequently evaporated under vacuum. TLC (section 3.7.2) was used to validate the purity of radioactive methotrexate and also to verify the identity of the labelled substance permeating through human stratum corneum. Methotrexate appeared as a blue spot (R_f 0.28) when viewed under UV light on the thin layer plate using n-butanol:pyridine:water (1:1:1) as the mobile phase. Methotrexate was found by this method to be 98% pure.

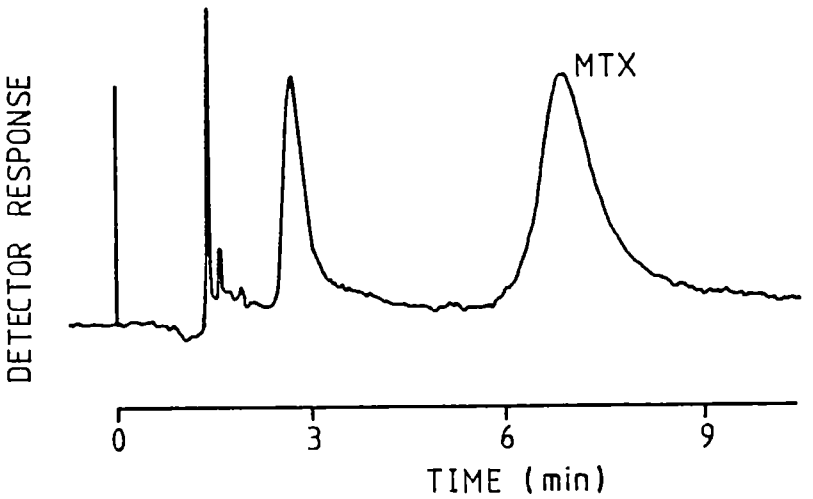
3.8.2 Steroids and other substances

Steroids and other radioactive substances were found to be ~98% pure using TLC (section 3.7.2).

3.9 Amount in the stratum corneum

3.9.1 Mass balance

Steady state cells (Figure 3.1) were used for this study. The cells were assembled (section 3.4) and placed in a water bath at 25°C. Samples were withdrawn simultaneously from both the donor and receptor compartments at regular intervals. The amount of solute retained in the stratum corneum was then calculated by mass balance (section 2.4) after determination of the amounts in the donor and receptor compartments.



HPLC CONDITIONS

Column:	0·01mm Phenyl μBondapak, 300mm × 3·9mm
Mobile phase:	11% acetonitrile in water, pH 4 6
Flow-rate:	2 ml / min
Detector:	Fixed UV, λ=313nm, 0·01 AUFS with 10mv recorder.

Figure 3.7 A chromatogram obtained during HPLC purification of methotrexate. Fraction of purified methotrexate was collected between 6 and 9 minutes.

3.9.2 Steady state

The amount in the stratum corneum at steady state was also determined at the end of each permeation run for steroids and methotrexate. At the end of each run the permeation cells were dismantled and the superficial substance on the exposed area was removed by washing. The stratum corneum was then immersed in fresh receptor fluid in a amber coloured bottle (without any agitation). The desorption fluid was replaced a number of times over a period of about 72 hours until it gave a count or absorbance of zero. The total amount was calculated by using the counts or absorbance of the pooled desorption solutions. The concentration in the stratum corneum, for methotrexate and steroids only, was also determined by using the weight of 4.5 cm^2 of the stratum corneum (72 $\pm$ 0.02 mg; n = 4).

3.10 Integrity of the stratum corneum

The integrity of the stratum corneum was examined at the end of each permeation run by quantifying the permeation of tritiated water (k_p= 1-9 x 10^{-7} cm sec^{-1}) over a period of 2-3 hours. The tritiated water (0.1 ml) was analysed by liquid scintillation counting using 10 ml of biofluor (section 3.7.4). Visual examination using congo red was also carried out to examine the integrity of the stratum corneum.

3.11 Estimation of solubility and partition coefficients

Excess solute was shaken intermittently with a solvent in a glass stoppered container at 25°C for approximately three days. The

saturated solution was then filtered through a 0.45 micrometre millipore filter. The solubilty was estimated by analysing the filtrate spectrophotometrically or by HPLC.

The method of Hansch (1973) was used to measure the octanol/water partition coefficients for the solutes as follows. Several concentrations of suitable volumes of solute and water-saturated octanol were tumbled for twenty four hours at 25°C. At equilibrium both octanol and water phases were analysed using the appropriate analytical procedure (Table 3.1).

3.12 Validation of the experimental techniques

Preliminary permeation experiments were carried out, to validate the reproducibility of the experimental technique, by measuring the permeation of phenol, chlorocresol and hydrocortisone through human stratum corneum (sections 3.3 and 3.4; Table 3.1).

3.12.1 Sampling

Ten samples of water were simultaneously withdrawn and weighed on the analytical balance. The reproducibility in the volume of a sample withdrawn ($\pm$ SD, n = 10) from the permeation cell was found to increase with the reduction in the volume measured, 0.1 $\pm$ 0.02 ml in comparison to 0.5 $\pm$ 0.01 ml or 1 $\pm$ 0.003 ml, using micropipettes.

3.12.2 Variation between permeation cells on different days

Phenol was chosen to carry out a series of permeation experiments which will allow the examination of possible variations between cells on different days.

After two halves of each individual cells were paired and tested for leakage, the method described in section 3.4 (Table 3.1) was followed to measure the cumulative amount of phenol in the receptor compartment. The permeability coefficients (Table 3.3) were then calculated using equation 2.5.

3.12.3 Inter and intra-variations in specimen of skin

Because of the large number of permeation studies (> 500 individual permeation runs) it was necessary to use human skin samples from more than 80 cadavers during the course of this work. To minimize inter-specimen variations (section 2.3.2) and the frequent non-availability of the skin samples it was possible only to conduct duplicate permeation experiments for the majority of steroids and weak electrolytes.

3.13 Preliminary experimental results

It was found that the permeability coefficients of phenol, chlorocresol and hydrocortisone were of the same order as reported previously (Scheuplein et al 1969; Roberts et al 1977).

An analysis of variance (Table 3.4) shows there is no significant difference in the rate of permeation between cells nor is there any significant difference in the rate of permeation in any one cell on different days (Figure 3.1). No such studies were carried out for non-steady state cells as the glass cells were made of the same material and the permeation studies were carried out under the same conditions.

The permeabilty coefficient of phenol was also used to compare

Table 3.3 Permeability coefficients [cm min^{-1}] x 10^4 of phenol, in between steady state cells* on different days.

Number of cell	Day 1	Day 2	Day 3
1	1.95	1.50	1.33
2	1.50	1.68	1.75
3	1.63	1.80	1.88
4	1.75	1.88	1.50
5	1.80	1.63	1.82
6	1.90	1.70	1.85

* Similar assembly was used for non-steady state cells.

Table 3.4 Validity of the experimental technique for steady state cells [Figure 3.1]

Analysis of variance [randomised complete block design] using the data shown in Table 3.3.

Source of variance	Degrees of Freedom	Sum of squares	Mean square	F-ratio	F(pr:0.05)
Between cells	5	0.18	0.036	1.71	3.33(df5/10)
Between days	2	0.10	0.05	2.38	4.10(df2/10)
Residual	10	0.21	0.021	-	-
Total	17	0.49	-	-	-

* from the F-table.

the inter and intra-specimen variations of the skin. It was found that inter-specimen variations was higher (coefficient of variation of about 15%, n = 10) than intra-specimen variation (coefficient of variation of about 5%, n = 6) for the human abdominal skin.

Tables 3.5 and 3.6 show the amount of phenol and chlorocresol obtained by mass balance in the stratum corneum, respectively, using steady state permeation cell (Figure 3.1). The amounts obtained for both the solutes were found to fluctuate with time and did not follow any general pattern. These results show that the amount of ingredient penetrating the stratum corneum is so small that it equals or exceeds the errors in analysis (sections 2.4 and 3.12.1). Attempts to reduce the receptor volume so as to increase the cumulative amount permeating had no effect on the fluctuation of the amount obtained in the stratum corneum by mass balance. It was consequently considered that mass balance was not an appropriate and reliable means of estimating the amount in the stratum corneum. The amount in the stratum corneum was therefore measured at steady state for methotrexate and for steroids (section 3.9.2).

Table 3.5 Amount in the stratum corneum by mass balance for 0.4% phenol with 16.120 mg as the initial donor amount.

Time (minutes)	Amount in donor (mg)	Cumulative amount in receptor (mg/4.5 cm^2)	Amount in stratum corneum (mg)
3	14.681	0.000	1.439
5	14.339	0.001	1.780
8	13.861	0.001	2.258
10	15.022	0.001	1.097
20	14.681	0.030	1.409
30	14.681	0.054	1.386
60	14.544	0.144	1.432
90	13.827	0.234	2.059
150	13.725	0.411	1.984
210	13.691	0.585	1.844
350	13.827	0.939	1.354

Table 3.6 Amount in the stratum corneum by mass balance for 0.2% chlorocresol with 6.2 mg as the initial donor amount.

Time (minutes)	Amount in donor (mg)	Cumulative amount in receptor (mg/4.5 cm^2)	Amount in stratum corneum (mg)
3	5.636	0.002	0.562
5	5.636	0.003	0.561
8	5.448	0.004	0.748
10	5.636	0.005	0.559
20	5.636	0.035	0.528
30	5.636	0.071	0.492
60	5.386	0.211	0.603
90	4.869	0.359	0.972
150	4.822	0.600	0.778
210	4.587	0.849	0.764
350	4.165	1.269	0.766

CHAPTER 4

Permeation of steroids through the human stratum corneum

Steroids are probably the most widely used group of non-electrolytes. Topical steroids are used in the management of various skin disorders (section 1.5.1) and a large number of studies have been carried out to examine the factors controlling the percutaneous absorption of steroids (sections 1.5.1 and 2.3). The mechanism by which the steroids penetrate the human skin has however received limited attention. Scheuplein et al (1969) and Blank and Scheuplein (1969) have suggested that the main route for the steady state percutaneous penetration is through the "intracellular" route rather than via "shunts". However, it was also suggested that for more polar steroids, which have long lag periods, that the "shunts" may be the major pathway at all times (Scheuplein et al 1969; Blank and Scheuplein 1969; section 2.2.1).

In order to separate the contributions of the "shunts" and "intracellular" routes, the inert membrane approach of Roberts and Anderson (1975) can be used. In this approach, the steady state fluxes of solutes through the skin and the inert membrane are compared and the relative contributions of drug/skin, vehicle/skin and drug/vehicle interactions are assessed (section 2.3.1.5). As the inert membrane is devoid of "shunts", it follows that the differences in the time course of solute transport through the human stratum corneum and the inert membrane can be used to ascertain the relative contributions of the "shunts" and "intracellular" routes to the percutaneous penetration of steroids.

This section of the work describes the experiments carried out to measure the *in vitro* rate of permeation of steroids through the human stratum corneum. Mathematical models have been used to determine the possible mechanism of epidermal transport of steroids through the excised human stratum corneum.

Theory

The transport of solutes through a membrane can be described by using mathematical equations derived from Fick's law of diffusion (Crank 1975; section 2.5). The diffusion approach or model is widely used in describing the transport of solutes through biological membranes and is characterized by a defined resistance barrier throughout the membrane (Crank 1975; Scheuplein 1978b).

The permeation of a solute through the stratum corneum can be related to permeation through a simple membrane provided it is assumed that the total diffusional resistance of the skin is due only to the stratum corneum and also that the physical characteristics are not altered by the application of the drug (Scheuplein 1978b).

(1) Simple diffusion through stratum corneum

When a constant concentration of a solute is applied to one side of a membrane and sink conditions maintained at the receptor side, the cumulative amount of solute (M) passing through the membrane from a constant concentration of solute (C_v) in time (t) may be related to the membrane/solute partition coefficient (K), the diffusion coefficient of solute in the membrane (D) and the

thickness of the membrane (h) by equation 4.1 (Crank 1975).

$$M = C_v Kh\left\{D/h^2 t - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp\left(-n^2\pi^2 Dt/h_2\right)\right\} \qquad \text{Eq. 4.1}$$

Equation 4.1 is subsequently referred to as diffusion model I or DM I. The parameters Kh and D/h^2 are often determined from the steady state portion of the curve of M versus t, the slope of the linear portion being the steady state flux (J_{ss}) and the intercept of the line on the x-axis being the lag time (Figure 2.3; section 2.5).

$$J_{ss} = k_p C_v = \frac{KDC_v}{h} = Kh \times D/h^2 \times C_v \qquad \text{Eq. 4.2}$$

where k_p is the permeability coefficient which can also be written as (section 2.5):

$$k_p = Kh \times D/h^2 \qquad \text{Eq. 4.3}$$

and lag time (t_L) can be expressed as (Crank 1975; section 2.5):

$$t_L = h^2/6D \qquad \text{Eq. 4.4}$$

(2) Transport through shunts

The permeation of steroids through "shunts" is characterized by fast diffusion coefficients and small fluxes, the latter reflecting a small surface area occupied by the "shunts"

(Scheuplein 1978b; Barry 1983; section 2.2.1). Hence, in this analysis the lag time for the transport of steroids through "shunts" is assumed to be negligible (Figure 4.1). The cumulative amount of solute (M_s) penetrating the skin via "shunts" is therefore given by:

$$M_s = C_v k_p t \qquad \text{Eq. 4.5}$$

(3) Total transport through stratum corneum

If the solute penetrates the skin through the "intracellular" route and the "shunts", the total amount transported is given by summing equations 4.1 and 4.5.

Consequently the cumulative amount of diffusant (M) per unit area that passes through the stratum corneum by both routes of skin penetration is given by:

$$M = \underbrace{C_v K_e h_e \left\{ D_e h_e^2 - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp\left(n^2 \pi^2 D_e t / h_e^2\right) \right\}}_{\text{"intracellular" route}} + \underbrace{C_v k_{p_s} t}_{\text{"shunts"}} \qquad \text{Eq. 4.6}$$

Equation 4.6 is subsequently referred to as diffusion model II or DM II.

4.1 Experimental

In vitro permeation cells used for steady state and non-steady permeation studies are described in sections 3.3.1 and 3.3.2 respectively. The permeation studies on seven steroids (Table 3.1) were carried out using the method described in section 3.4. The steady state flux and lag time was estimated by using the

method described in section 2.5 (Figure 2.3), by carrying out linear regression of the steady state portion of the data points. Permeability coefficients were then calculated using equation 2.5. Non-linear regressions (unweighted) were also carried out using equations 4.1 (DM I) and 4.6 (DM II), and data points of the steroids (section 3.7.5). The permeability coefficients and lag times were then estimated using the final parameter values of the diffusion models and equations 4.3 and 4.4 respectively.

4.2 Results

Diffusion model regression

Figure 4.1 shows the theoretical lines predicted by diffusion model II for different extents of "shunt" transport. As the "shunt" transport component increases the lag time becomes shorter. Figure 4.1b shows that the residuals of the regression line obtained using diffusion model I for 60% "shunt" data are not randomly distributed and that there is a systematic deviation in the residuals. A similar residual plot was obtained for 20% "shunt" data using diffusion model I. Hence, a systematic deviation of residuals may be anticipated with diffusion model I if a significant proportion of the solute is transported via the "shunts".

Figures 4.2 and 4.3 show the permeation of steroids through the human stratum corneum and the inert membrane respectively with the non-linear regression lines, derived using diffusion model I, drawn through the data points. Figure 4.4 shows a typical residual plot of the regression data obtained for the permeation

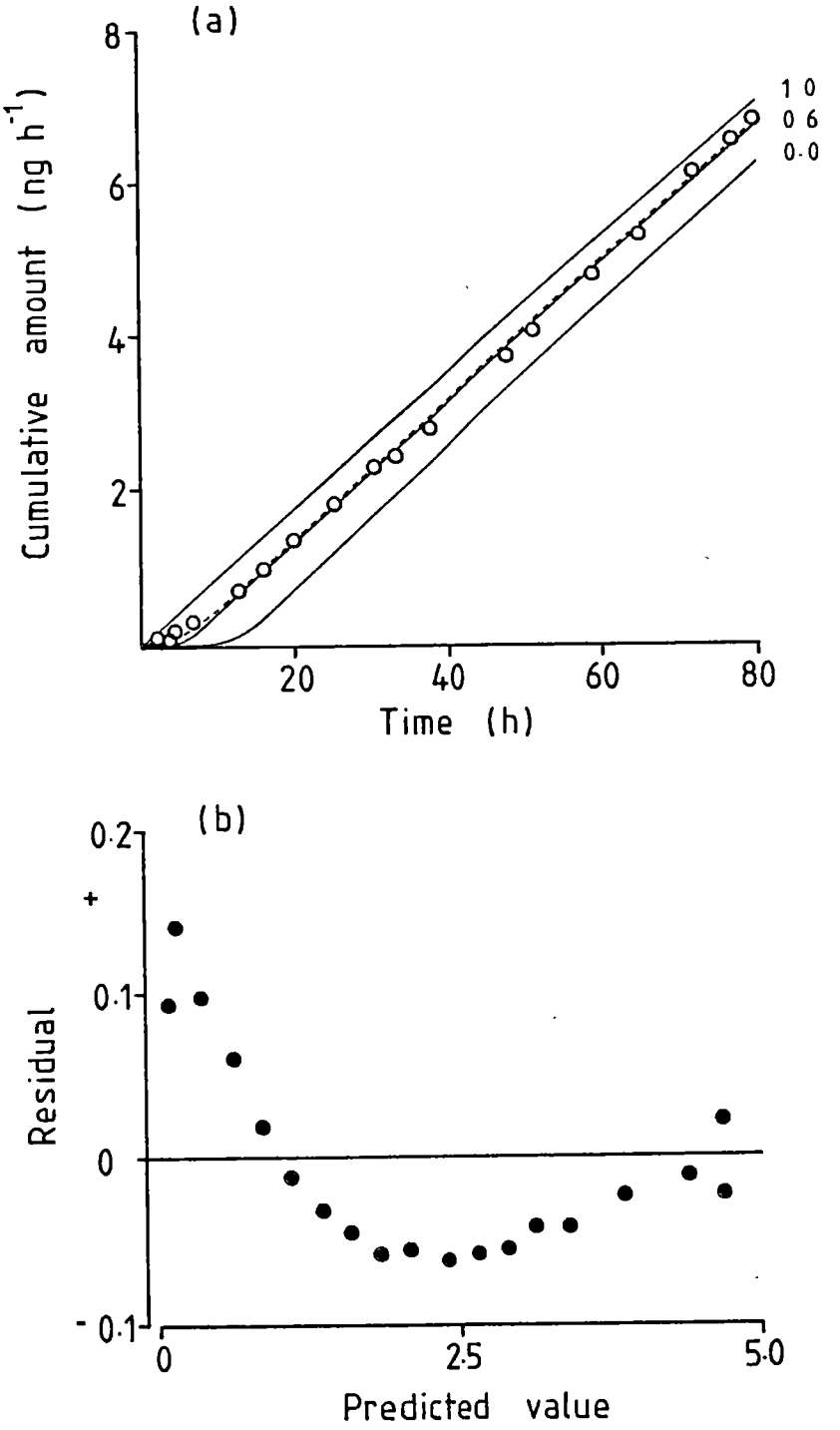


Figure 4.1 (a) Theoretical plots showing the increase in lag time with a decrease in the proportion of solute penetrating through the "shunts" (1.0, 0.6 and 0.0 are the fraction k_p "shunts"). The symbols represent the data points (o) for 60% shunt transport and the broken line is the result predicted by DM I. (b) Residual plot of the regression line predicted by DM I.

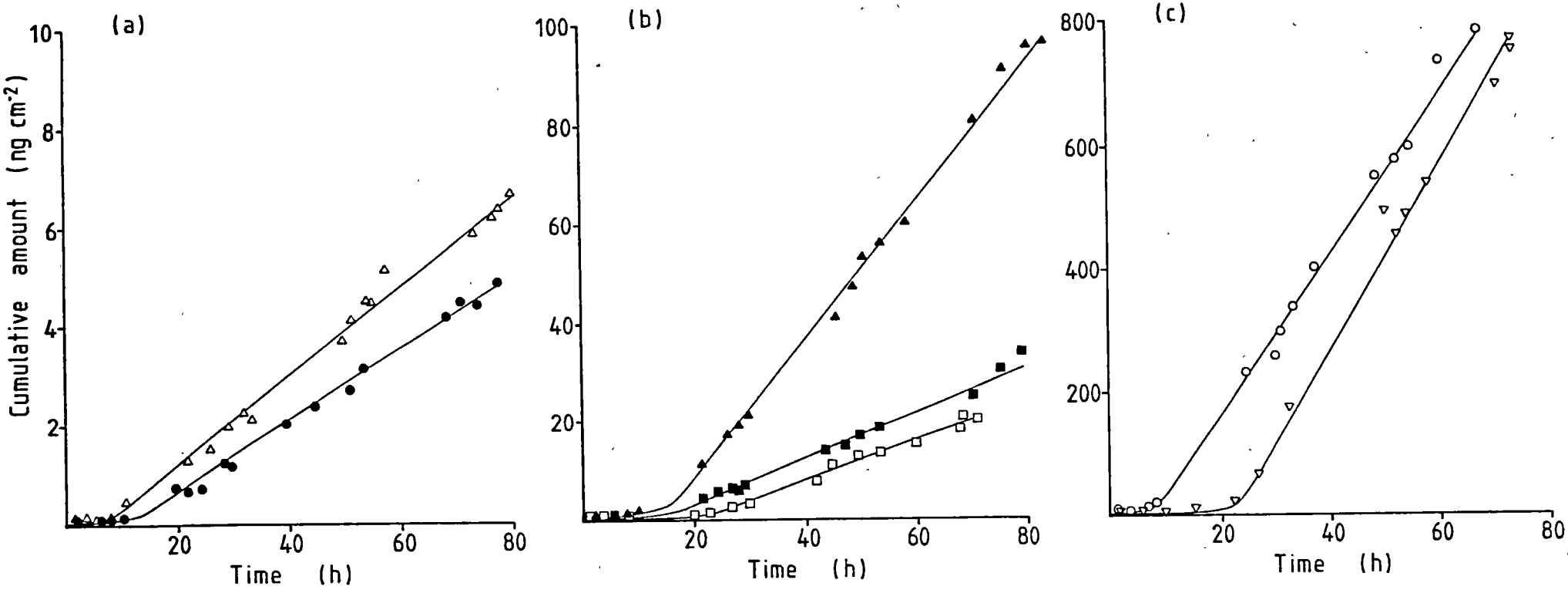


Figure 4.2 Permeation of steroids through the human stratum corneum. The symbols represent the experimental data and the solid line is the result predicted by DM I. (a) ● Hydrocortisone and △ Triamcinolone. (b) □ Triamcinolone acetonide, ■ Corticosterone and ▲ Prednisolone. (c) ▽ Testosterone and ○ Betamethasone 17-valerate.

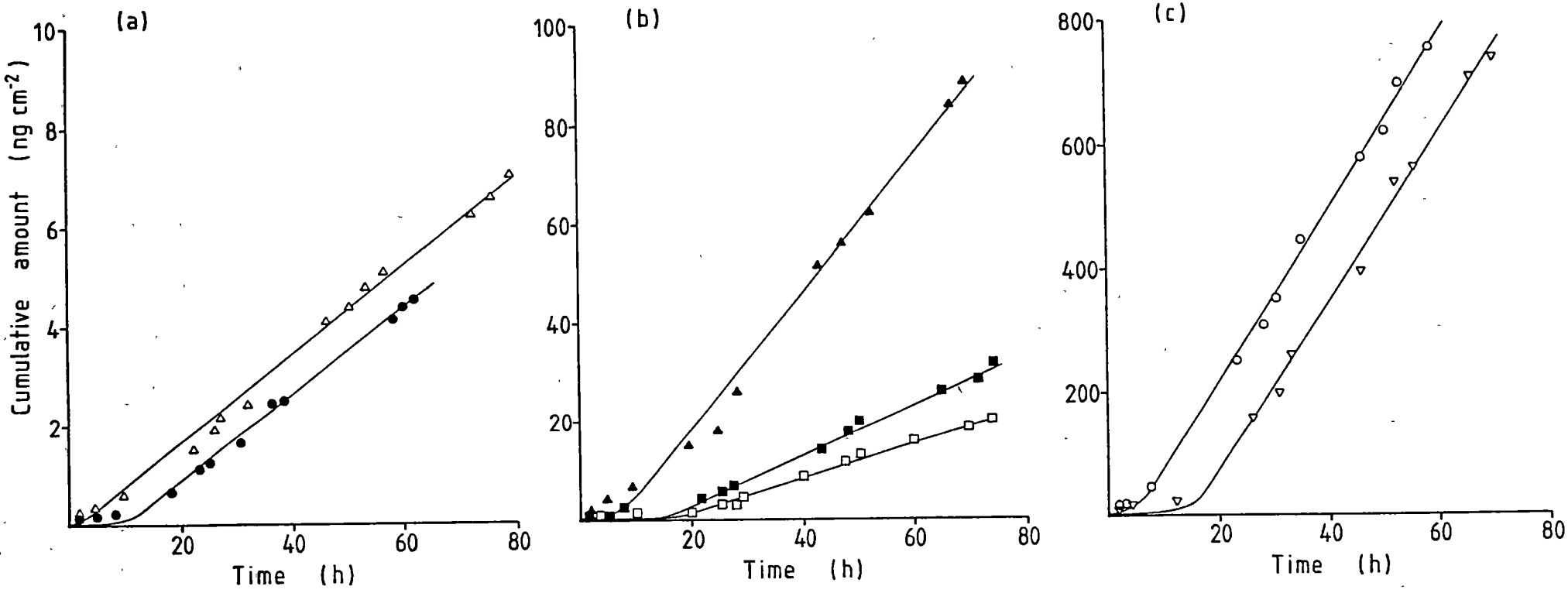


Figure 4.3 Permeation of steroids through the inert membrane. The symbols represent the experimental data and the solid line is the result predicted by DM I. (a) ● Hydrocortisone and △ Triamcinolone. (b) □ Triamcinolone acetonide, ■ Corticosterone and ▲ Prednisolone. (c) ▽ Testosterone and ○ Betamethasone 17-valerate.

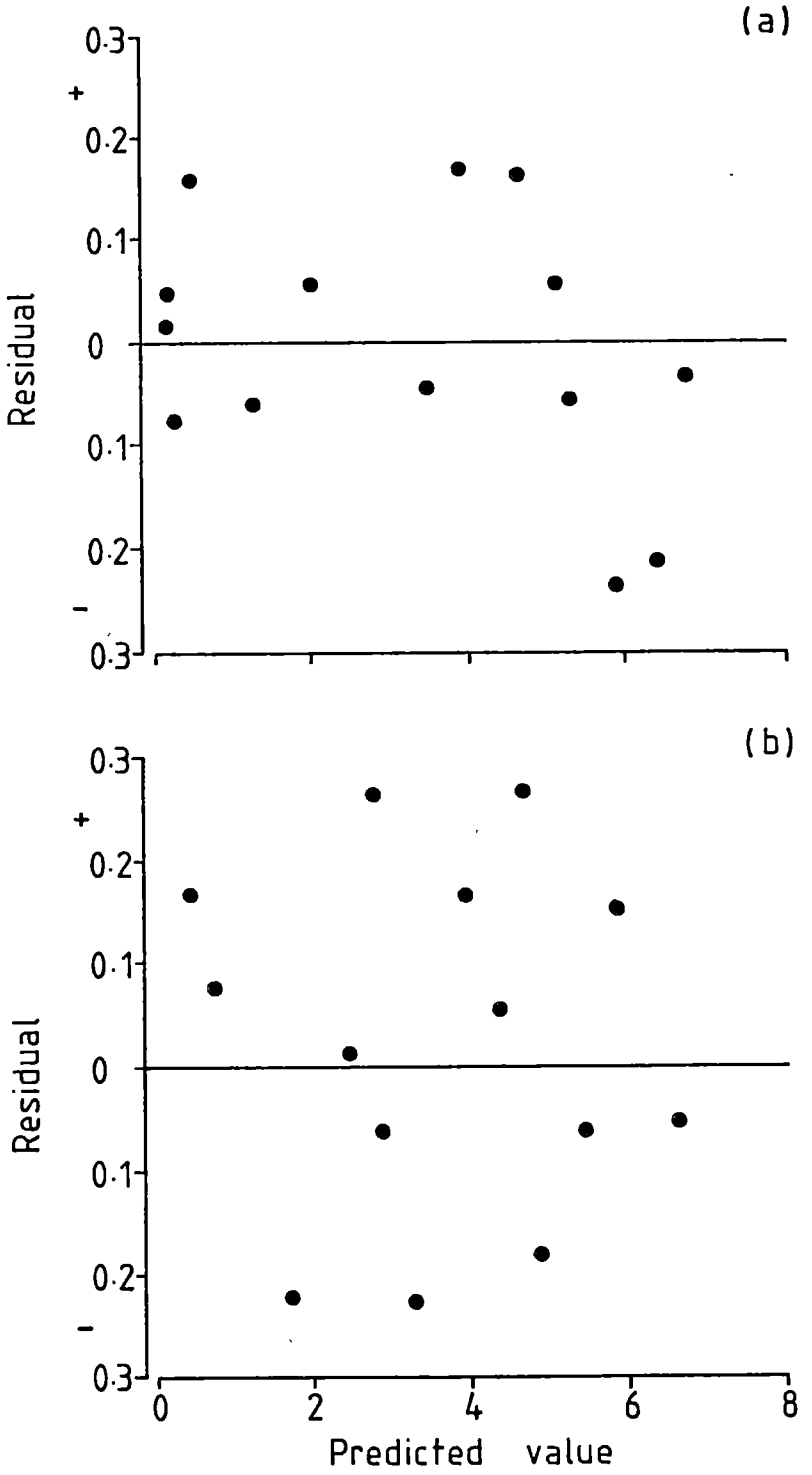


Figure 4.4 Residual plots (DM I) for the permeation of triamcinolone through the (a) human stratum corneum and (b) inert membrane.

of triamcinolone through the human stratum corneum and the inert membrane using diffusion model I. The residual plots for other steroids were also found to be randomly distributed.

Table 4.1 shows the final parameter values predicted by diffusion model I for the permeation of steroids through the human stratum corneum and the inert membrane (Figures 4.2 and 4.3). The coefficient of variation (%CV) for the final parameter values were found to be less than 30% for the majority of the steroids.

Table 4.2 shows the permeability coefficients predicted by diffusion models I and II. Regression fits similar to those of DM I (Figure 4.2) were also obtained for DM II. However, the model (DM II) indicates that the contribution of "shunt" transport is very small and also that the %CV's for the permeability coefficients for "shunts" were predicted to be higher than those of the "intracellular" route (Kh and D/h^2).

In vitro rate of perfusion

Figure 4.5 shows a typical plot of the effect of the in vitro perfusion rate on the rate of permeation of a polar steroid (triamcinolone) and a non-polar steroid (testosterone) through the human stratum corneum. The figure shows that the amount permeated increases with the increase in the rate of perfusion. The plots also show that at early times all data points appear to coincide while diverging at later times with increase in the flux and the attainment of the steady state penetration. At high flow rates (30 and 40 ml hr^{-1}) unusual cumulative-amount time profiles were observed for the majority of steroids used in the present

Table 4.1 Final parameter values for the permeation of steroids through the human stratum corneum and the inert membrane using diffusion model I [equation 4.1].

Steroids	Human stratum corneum				Inert membrane			
	N	Kh $\times 10^3$	D/h^2 (cm^2/sec) $\times 10^6$	r	N	Kh $\times 10^3$	D/h^2 (cm^2/sec) $\times 10^5$	r
Hydrocortisone	14	0.25 (11.98)	3.10 (8.65)	0.998	12	0.19 (15.45)	0.45 (12.110)	0.998
Triamcinolone	15	0.18 (27.56)	6.20 (23.99)	0.995	14	0.77 (58.02)	1.40 (54.750)	0.995
Triamcinolone acetonide	15	3.30 (11.21)	1.70 (6.41)	0.998	12	1.60 (20.09)	0.27 (13.930)	0.994
Corticosterone	16	2.20 (14.39)	2.80 (10.44)	0.997	12	1.80 (2.67)	0.33 (0.001)	0.993
Prednisolone	17	4.60 (9.96)	2.70 (7.18)	0.998	11	2.30 (34.17)	0.49 (28.060)	0.997
Testosterone	12	117.00 (18.91)	2.00 (11.83)	0.995	11	61.00 (27.05)	0.31 (20.330)	0.995
Betamethasone 17-valerate	14	70.00 (15.29)	5.60 (12.65)	0.998	11	40.00 (24.97)	1.10 (22.440)	0.998

The numbers in parenthesis are the %CV's.

Table 4.2 A comparison of permeability coefficients predicted by diffusion model I [DM I - equation 4.1] and diffusion model II [DM II - equation 4.2] for the permeation of steroids through the human stratum corneum.

Steroids	DM I*	Diffusion model II (DM II)			Fraction
	kp_e (cm/hr) x 10^6	Kh x 10^3	D/h^2 (cm^2/sec) x 10^6	kp_e (cm/hr) x 10^6	kp_s (cm/hr) x 10^6	shunts+
Hydrocortisone	2.82 (10.64)	0.26 (14.54)	3.00 (18.52)	2.81 (10.68)	0.16 (172.30)	0.05
Triamcinolone	3.98 (45.23)	0.18 (30.42)	5.50 (64.38)	3.60 (50.00)	0.38 (482.20)	0.09
Triamcinolone acetonide	20.20 (4.95)	3.30 (11.21)	1.62 (6.43)	19.25 (5.20)	0.40 (140.00)	0.02
Corticosterone	22.39 (8.49)	3.00 (40.15)	1.32 (33.70)	14.10 (23.40)	7.00 (30.00)	0.33
Prednisolone	44.67 (7.39)	5.00 (14.34)	2.30 (18.26)	42.00 (5.20)	5.00 (76.77)	0.11
Testosterone	851.14 (4.70)	134.00 (25.69)	1.60 (14.77)	772.00 (5.18)	84.00 (82.00)	0.10
Betamethasone 17 - valerate	1148.15 (25.72)	69.00 (16.38)	5.30 (17.72)	1311.00 (22.53)	6.90 (30861.00)	0.01

*kp_e (DM I) was calculated using the values shown in Table 4.1.

The numbers in parenthesis are the %CV.

+Fraction shunts (kp_s) = $kp_s/kp_s + kp_e$

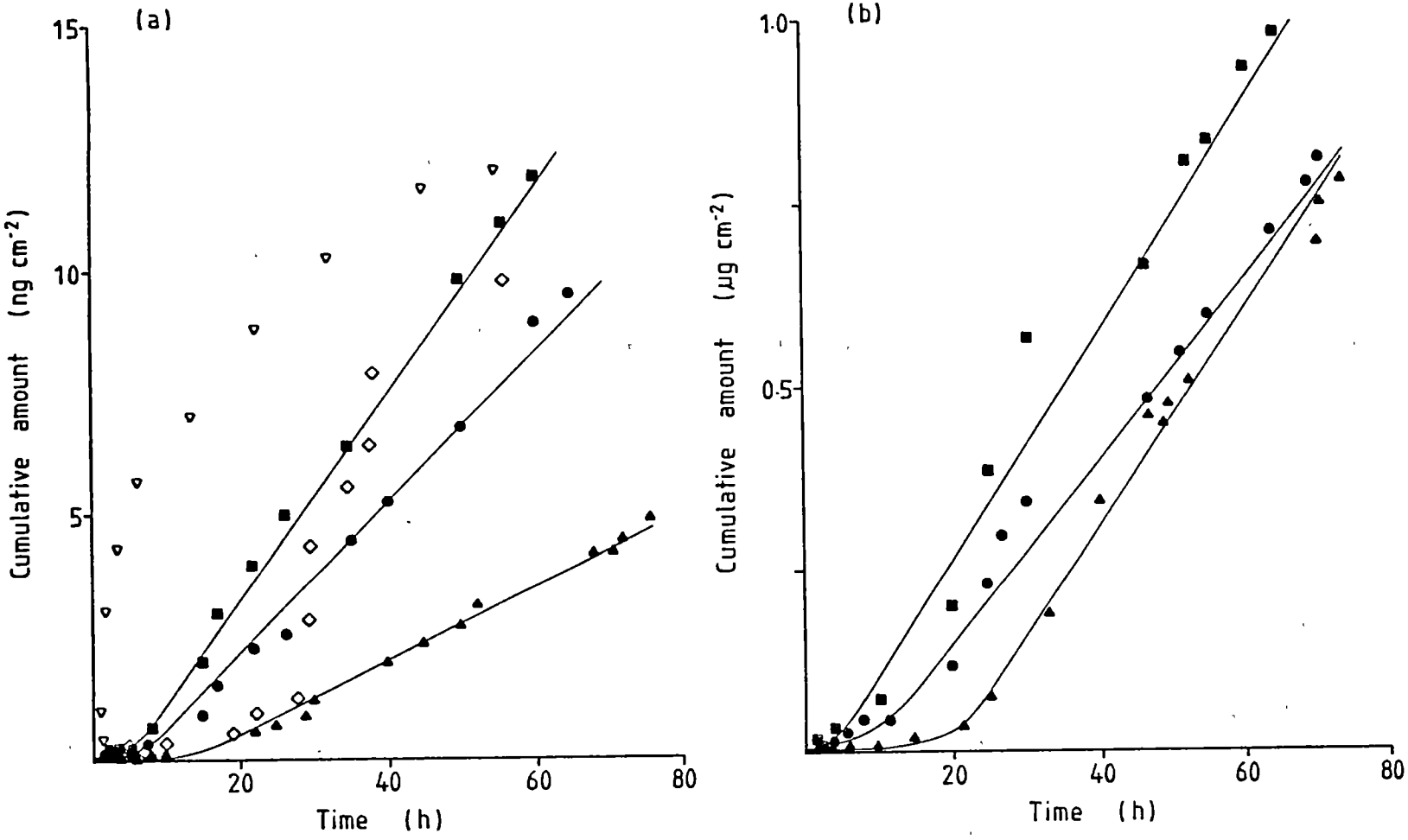


Figure 4.5 Effect of <u>in vitro</u> rate of perfusion on the permeation of aqueous steroid solution through the human stratum corneum. The solid line drawn through the data points of (a) Triamcinolone and (b) Testosterone for 0, 10 and 20 ml/hr is the result predicted by diffusion model I. ▲ 0 ml/hr, ● 10 ml/hr, ■ 20 ml/hr, ◇ 30 ml/hr, ▽ 40 ml/hr.

work. Therefore the data obtained for the perfusion rates of 0, 10 and 20 ml hr^{-1} was only used to estimate the permeability coefficients of steroids.

Table 4.3 shows the effect of the in vitro rate of perfusion on the permeation of aqueous steroid solutions through the human stratum corneum. The permeability coefficients for the majority of steroids were found to increase with increase in flow rate in the receptor compartment, except for triamcinolone acetonide. Lag times generally showed a small decrease with increase in the flow rate.

Type of the receptor fluid

The rate of penetration of steroids did not depend upon the type of receptor fluid used in the steady state cell (Figure 3.1). The rate of penetration of steroids only increased by 2-5% when aqueous protein solution (SPPS) was used in the receptor compartment (Figure 3.1) instead of isotonic sodium chloride.

Partition coefficients

Figure 4.6 shows that the permeability coefficients for the human stratum corneum were related to the octanol/water partition coefficients of the steroids. The permeability coefficients of steroids for the inert membrane were also found to increase with increase in the octanol/water partition coefficients (Tables 4.4 and 4.5).

It was also found that there was a direct linear relationship between the log kp of steroids through the human stratum corneum

Table 4.3 Effect of in vitro rate of perfusion on the permeability coefficients and lag times of steroids.

Steroids*	kp x 10^6 (cm hr^{-1})			lag times (hrs)		
	0 ml hr^{-1}	10 ml hr^{-1}	20 ml hr^{-1}	0 ml hr^{-1}	10 ml hr^{-1}	20 ml hr^{-1}
Hydrocortisone	2.82 ±0.3	6.00 ±0.7	7.50 ±0.9	14.93 ±0.5	11.19 ±0.8	5.56 ±1.0
Triamcinolone	3.98 ±1.8	6.40 ±0.5	6.60 ±0.7	7.47 ±2.0	6.95 ±1.5	1.50 ±3.0
Triamcinolone acetonide	20.20 ±1.0	13.70 ±3.0	19.60 ±0.8	7.50 ±0.5	8.00 ±0.8	7.00 ±1.0
Corticosterone	22.39 ±1.9	24.00 ±0.7	29.00 ±0.9	16.53 ±1.5	14.62 ±1.4	12.00 ±1.8
Prednisolone	44.67 ±3.3	48.00 ±1.0	50.00 ±1.2	17.15 ±2.4	11.20 ±3.0	8.80 ±2.2
Testosterone	851.14 ±40.0	900.00 ±44.0	960.00 ±90.0	23.15 ±3.0	8.10 ±4.2	2.70 ±3.5
Betamethasone 17-valerate	1148.15 ±295.4	1400.00 ±200.0	1650.00 ±350.0	8.27 ±2.2	4.50 ±1.8	4.70 ±1.9

*± SD, kp and lag times predicted by a diffusion model I.

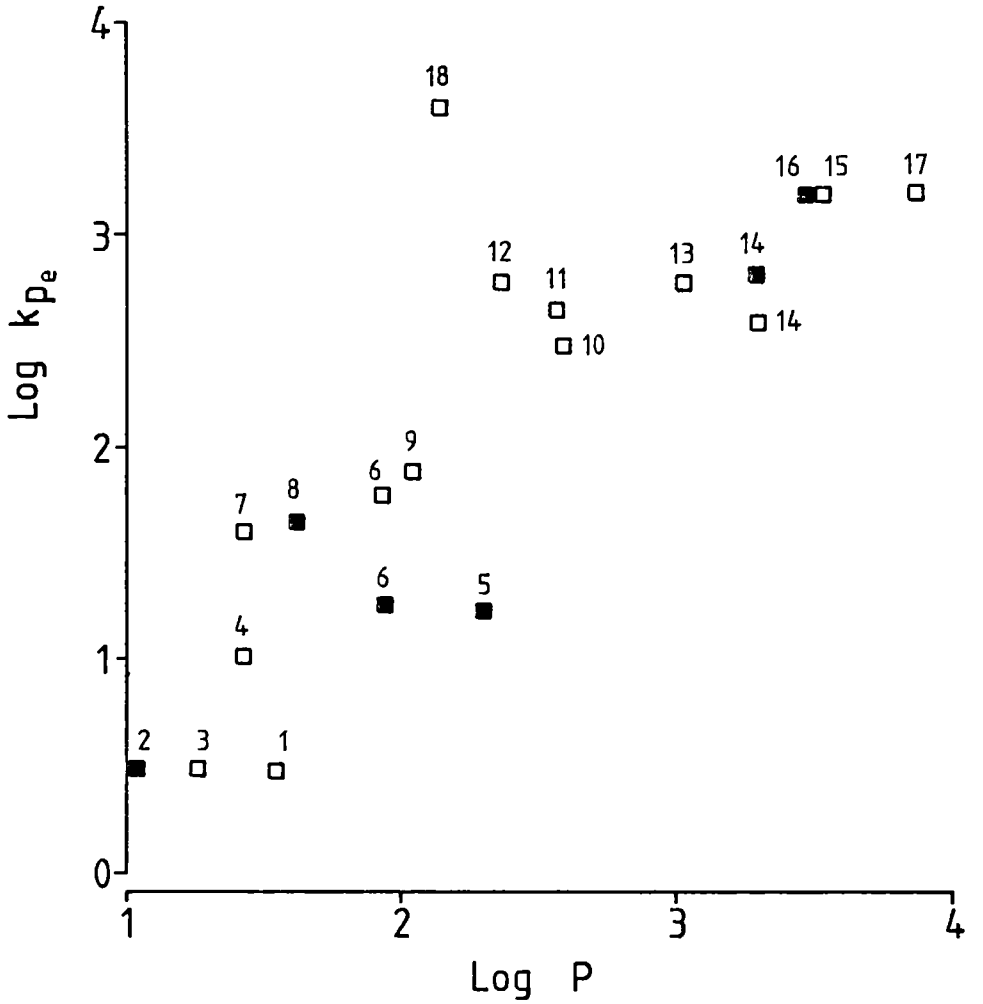


Figure 4.6 Relationship between permeability coefficient (linear regression) of human stratum corneum (k_{pe}) and octanol/water partition coefficient of steroids. Numbers are for different steroids shown in Table 4.4.
■ k_p (experimentally determined) and
□ k_p (Scheuplein et al. 1969).

Table 4.4 Permeability coefficients (kp), lag times, octanol/water partition coefficients (P) and concentrations in the stratum corneum at steady state ($C_{st.c}$) for steroids.

Steroids	log (kp x 10^6) cm hr^{-1}		lag time hrs		log P	$C_{st.c}$ $\mu g\ ng^{-1}$
	human skin	inert membrane	human skin	inert membrane		
(1) Hydrocortisone	0.45	0.49	14.9	10.3	1.55[b]	1.20
	0.48[a]					
(2) Triamcinolone	0.60	1.59	7.5	3.3	1.03[b]	1.21
(3) Aldosterone	0.48[a]	-	-	-	1.25[c]	-
(4) Cortisone	1.00[a]	-	-	-	1.42[b]	-
(5) Triamcinolone acetonide	1.30	1.20	27.2	17.2	2.31[b]	1.53
(6) Corticosterone	1.35	1.33	16.5	14.0	1.94[b]	1.30
	1.78[a]					
(7) Estriol	1.60[a]	-	-	-	1.43[c]	-
(8) Prednisolone	1.65	1.60	17.2	9.5	1.62[b]	1.24
(9) Cortexolone	1.88[a]	-	-	-	2.04[c]	-
(10) Estradiol	2.48[a]	-	-	-	2.59[c]	-
(11) Cortexone	2.65[a]	-	-	-	2.58[c]	-
(12) Hydroxyprogesterone	2.78[a]	-	-	-	2.36[b]	-
(13) Hydroxypregnenolone	2.78[a]	-	-	-	3.03[c]	-
(14) Testosterone	2.93	2.83	23.2	14.9	3.29[b]	1.95
	2.60[a]					
(15) Betamethasone 17-valerate	3.06	3.20	8.3	4.2	3.49[b]	1.92
(16) Pregnenolone	3.20[a]	-	-	-	3.54[c]	-
(17) Progesterone	3.20[a]	-	-	-	3.87[b]	-
(18) Estrone	3.60[a]	-	-	-	2.14[c]	-

Values shown in Table 4.1 were used to calculate kp and lag times.
(a) - Scheuplein et al (1969)
(b) - Valvani and Yalkowsky (1980)
(c) - Roberts et al (1977b)

Table 4.5 Relationship between the physicochemical properties of steroids and its permeation through the human stratum corneum (e) or the inert membrane (m).

Plots	slope	intercept	r
$\log k_{p_e}$ versus log P	0.69	0.93	0.83
$\log k_{p_m}$ versus log P	0.78	0.82	0.80
$\log k_{p_e}$ versus $\log k_{p_m}$	0.85	0.37	0.93
t_{L_e} (0 ml/hr) versus t_{L_m}	1.28	3.10	0.95
t_{L_e} (10 ml/hr) versus t_{L_m}	0.29	6.25	0.45
t_{L_e} (20 ml/hr) versus t_{L_m}	0.29	2.90	0.45

and the inert membrane and also between the lag times for both types of membranes (Table 4.5). However, the lag times were generally found to be shorter for the inert membrane on comparison with the human stratum corneum (Table 4.4).

The concentrations of the steroids retained in the stratum corneum were found to increase with decrease in the polarity of the steroids (Table 4.4).

Importance of data weighting in non-linear regression

The final parameter values and the residual plots obtained by diffusion model I for the simulated data of 60% "shunts" (Figure 4.1), and for the permeation of triamcinolone and testosterone through the human stratum corneum (Table 4.1) were independent of the type of weighting used during the non-linear regression. The final values predicted by diffusion model I without weighting, were identical to those predicted by the same model when a y^{-1} or y^{-2} or $y^{-1/2}$ weighting factor was used.

4.3 Discussion

Mechanism of epidermal transport

The present work has shown that the permeation of steroids through excised human stratum corneum, and also through an inert membrane, from aqueous solutions can be adequately described by a simple diffusion model, DM I, (Figures 4.2 and 4.3). The data points for the steroids were found to be randomly distributed around the regression lines. An example of the residual distribution of triamcinolone is shown in Figure 4.4. The

permeability coefficients predicted by diffusion model I are of a similar order to those reported by Scheuplein et al (1969) for the same compounds (Table 4.4).

The permeability coefficients for the "intracellular" route (k_{pe}) predicted by diffusion model I were also of similar order to those predicted by diffusion model II (Table 4.2); in addition low values of permeability coefficients were obtained for the "shunt" transport which indicates that the contribution of the "shunt" pathway to the total transport through the excised human stratum corneum was less than 11% in all cases.

The value of the permeability coefficients for "shunts" predicted by diffusion model II was found to be independent of initial parameter estimates. For example the final permeability coefficient value for "shunts" (kp_s) predicted for triamcinolone (3.8×10^{-7} cm hr^{-1}) was found to remain constant when initial estimates of 3.8×10^{-5} or 3.8×10^{-6} or 3.8×10^{-7} cm hr^{-1} were used. This shows that the low values of the permeability coefficients (kp_s) and kp_e) predicted by diffusion model II were not influenced by the value of the initial parameter used during the non-linear regressions.

The results indicate that the bulk transport of steroids through excised human stratum corneum occurs via the "intracellular" route with a negligible contribution (less than 10%) by "shunts" during the transient period (non-steady state) of skin penetration (Table 4.2). This hypothesis is further validated by the comparatively fast penetration of steroids through the inert membrane (Table 4.4). The ease with which steroids penetrate the

inert membrane, with short lag times, and taking into account the small fractional area of "shunts" in human skin (section 2.2.1), it follows that it is more likely that the bulk transport of polar steroids occurs through excised human stratum corneum via the "intracellular" route rather than through the "shunts".

The *in vitro* work of Scheuplein et al (1969) indicates that for the more polar steroids (hydrocortisone or triamcinolone), penetration through "shunts" may be important before a steady state is established. The penetration of hydrocortisone *in vivo* has been reported to achieve maximum flux within 24 hours (Feldman and Maibach 1967) and it has been suggested by Scheuplein et al (1969) that it may be possible that bulk transport of hydrocortisone occurs via "shunts". However, the lag times obtained for hydrocortisone in this work (5.56-14.93 hours, Table 4.3) suggest that it may also be possible to achieve a maximum flux within 24 hours via the "intracellular" route.

The contribution of "shunts" to the overall penetration of hydrocortisone through excised human stratum corneum was found to be approximately 5% (Table 4.2). The discrepancy between the present *in vitro* studies and the previous *in vitro* and *in vivo* work of Scheuplein et al (1969) and Feldman and Maibach (1967) respectively, could be due either to the scarcity of hair follicles in the abdominal area of the human body or to the hydration effect of the receptor fluid on the excised human stratum corneum during the *in vitro* experiments. In addition it is possible that the lumens of the sweat ducts which may act *in vivo* as "shunts", were deformed and swollen due to hydration

(section 2.3.1.1), during the in vitro experiments (Scheuplein et al 1969), thus only allowing the penetration of steroids through the "intracellular" route. It has also been pointed out by Scheuplein et al (1969) that the presence of a reservoir of steroid in the skin (section 2.2.2) is an indication of "shunt" transport during in vivo studies. However, the fast in vitro penetration of non-polar steroids through the excised human stratum corneum and the inert membrane (Table 4.4), and also the low fraction predicted by diffusion model II for "shunt" transport (Table 4.2), appears to be consistent with the pattern observed in in vivo experiments (Mckenzie 1962; Scheuplein et al 1969; Wickrema-sinha et al 1978), indicating that the bulk transport of non-polar steroids also occurs through the "intracellular" route both in vitro and in vivo.

Importance of polarity

The general increase in the stratum corneum (or inert membrane) permeability coefficients can be related to the decrease in polarity or increase in the partition coefficient of the steroids (Table 4.4, Figure 4.6). This could be due to the predominantly lipophilic nature of the human stratum corneum, which would facilitate the percutaneous penetration of highly lipophilic substances (Scheuplein et al 1969; Schaefer et al 1982). The increase in the rate of penetration is greatest for testosterone, betamethasone 17-valerate, pregnenolone and progesterone (Table 4.4), indicating that the highly non-polar nature of the steroids is likely to have a marked effect on percutaneous absorption. The high stratum corneum permeability coefficient found for

betamethasone 17-valerate in comparison with that of hydrocortisone confirms that the presence of lipophilic moieties of valerate would enhance the percutaneous absorption of corticosteroids (Katz and Shaikh 1965; Flynn 1979).

The solubility of the steroid in a solvent (sections 2.3.1.3 and 2.3.1.4) may be correlated with the rate of penetration through the stratum corneum (Scheuplein et al 1969). However, the concentrations of steroids used during this study were very small, the donor compartment having contained the steroids as aqueous solutions (Table 3.1; Valvani and Yalkowsky 1980). The amounts retained in the excised human stratum corneum were found to increase with decrease in polarity (Table 4.4). The difference in the amounts in the stratum corneum for polar and non-polar steroids is too small to draw any definitive conclusions. However, Scheuplein et al (1969) have reported that there was no relationship between the stratum corneum/steroid partition coefficients and the permeability coefficients of the steroids. On the other hand the increase in the permeability coefficients with the increase in the octanol/water partition coefficients of the steroids (Table 4.4; Figure 4.6) is in general agreement with the results reported by Scheuplein et al (1969).

Effect of in vitro rate of perfusion

The rate of penetration was found, with the exception of triamcinolone acetonide, to increase with increase in the in vitro rate of perfusion (Table 4.3). These results are in general agreement with those reported by Crutcher and Maibach (1969) for testosterone, and Bronaugh and Stewart (1985) for

cortisone. However, the effect of the in vitro perfusion rate on the rate of permeation appears to become less important with decrease in polarity (Tables 4.3 and 4.4). The increase in the permeability coefficient for triamcinolone was found to be approximately two fold higher in comparison to testosterone or betamethasone 17-valerate. The reason for the decrease in the permeability coefficient of triamcinolone acetonide with the increase in the rate of permeation is not known.

The concentration, or the cumulative amounts, recovered in the receptor compartment at a flow rate of greater than 20 ml hr^{-1} were found to fluctuate with time and did not follow any general pattern (Figure 4.5). It is suggested that, at a rate of less than 20 ml hr^{-1}, the flow of liquid through the permeation cell is in streamline; when the flow rate exceeds 20 ml hr^{-1} the flow becomes turbulent and unstable, as the Reynolds number for the permeation cell shown in Figure 3.2 is greater than 4 when the rate of perfusion is greater than 20 ml hr^{-1}, the critical Reynolds number being less than or equal to 4 (White 1974). The results obtained for the flow rates of 10 and 20 ml hr^{-1} were generally found to be consistent (Table 4.3). The problem of fluctuation of amounts (or concentration) with time was not encountered by Anjo et al (1980) for parathion at a flow rate of more than 20 ml hr^{-1}; this may be due to the design of their permeation cell, where the flow might have been less turbulent and more stable than in the present case. It has recently been reported by Bronaugh and Stewart (1985) that greater variability in the in vitro rate of permeation of substances was obtained at a perfusion rate of 40 ml hr^{-1} in comparison with 5 ml hr^{-1}, and

that the two flow rates showed almost identical rates of permeation through animal skin. This indicates that to carry out a permeation study at perfusion rates greater than 20 ml hr^{-1} it is important to design the permeation cells so as to minimize this problem.

The blood flow in the skin is approximately 0.05 ml min^{-1} per cubic centimeter at 23°C under normal conditions (Flynn 1979); thus for the permeation cell shown in Figure 3.2, a rate of *in vitro* perfusion of 10 ml hr^{-1} might mimic the *in vivo* conditions. It might be more appropriate to carry out *in vitro* perfusion studies only at a flow rate which would mimic the normal blood flow in the skin.

Lag times

The lag times were found to decrease with increase in the permeability coefficients (Table 4.4). The dependence of lag times on the rate of percutaneous penetration and the partition coefficient is in general agreement with that reported by Scheuplein et al (1969). The increase in the *in vitro* perfusion rate also reduced the lag times for all the steroids (Table 4.3). This reduction in the lag times with the increase in the perfusion rate could be due to the increase in the contribution of "shunts" to the overall penetration of steroids as it may be possible that the lipid fraction of the stratum corneum is partially removed during *in vitro* perfusion and this may create artificial "shunts" (Blank and Scheuplein 1969). The hydrostatic pressure on the skin during perfusion studies may also facilitate the creation of artificial "shunts" resulting in the fast rate of

penetration through the human stratum corneum. The high permeability coefficients and the low lag times obtained with increase in the perfusion rate may therefore possibly be due to the creation of artificial "shunts".

The above hypothesis could be validated by comparison of lag times for the inert membrane and the human stratum corneum at different rates of perfusion (Figure 4.7). There was some deviation between the lag times of the inert membrane and the stratum corneum at 10 and 20 ml hr^{-1}, as indicated by the low r-values (Figure 4.7 and Table 4.5). Diffusion model II showed a small increase in the values of the permeability coefficients of "shunts" at in vitro flow rates of 10 and 20 ml hr^{-1}. This increase in the permeability coefficient was very small (2-5%) and it is difficult therefore to deduce the significance of artificial "shunt" transport in the percutaneous penetration of steroids at in vitro flow rates of 10 and 20 ml hr^{-1} using a model approach.

Conclusions

The results presented in this section of the work suggest that the in vitro rate of percutaneous penetration of steroids depends upon their polarity. The ideal in vitro perfusion rate must be determined for the permeation cell, so that it might be possible to compare the in vitro data quantitatively with the in vivo data. However, a non-steady state cell with appropriate sink conditions would suffice when only qualitative interpretation of the data is required.

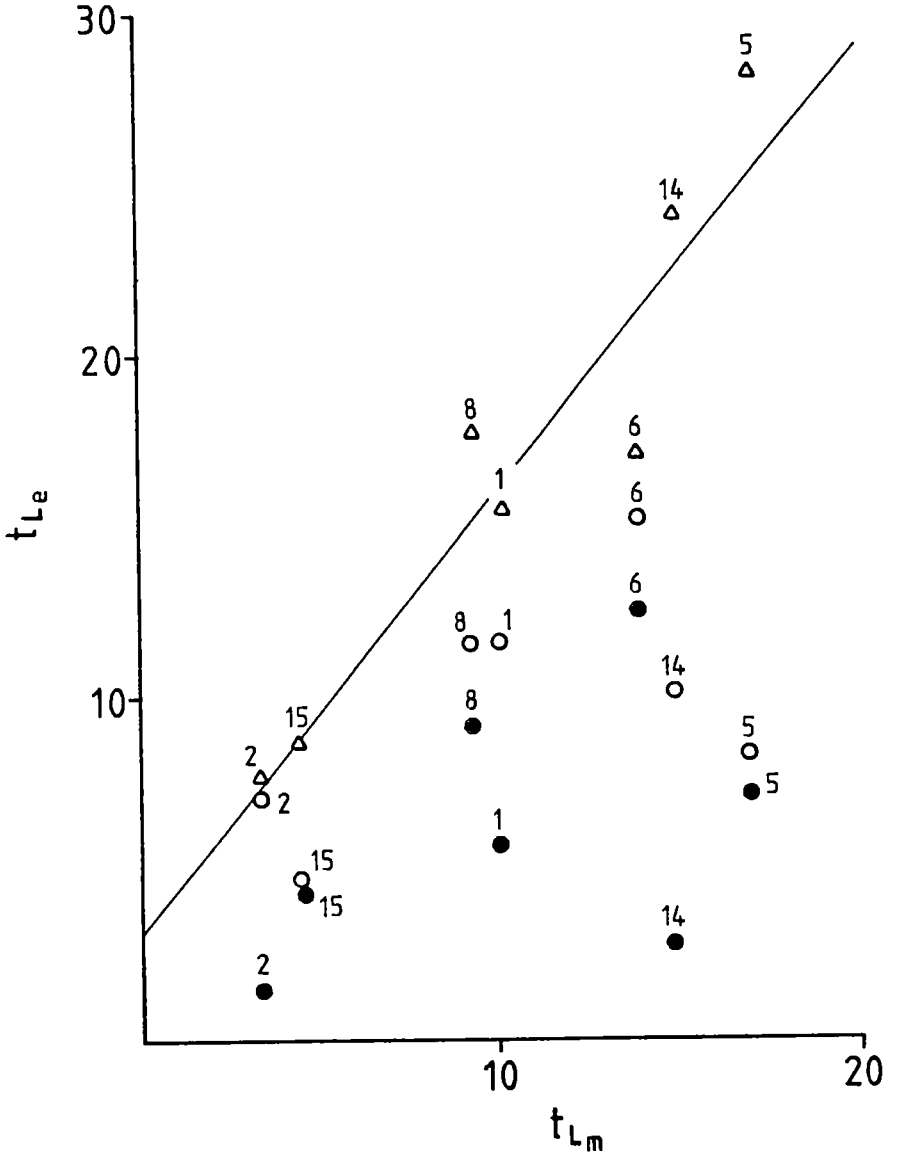


Figure 4.7 Relationship between the lag time for the inert membrane (t_{Lm}) and the stratum corneum (t_{Le}). Numbers are different steroids shown in table 4.4. The solid line is the best fit line for data of 0 ml/hr. △ 0 ml/hr t_{Le} versus t_{Lm}, ○ 10 ml/hr t_{Le} versus t_{Lm}, ● 20 ml/hr t_{Le} versus t_{Lm}.

The present results also suggest that the in vitro permeation of steroids through the excised human stratum corneum and the inert membrane could be adequately described by a simple diffusion model (DM I). Good fits obtained for the excised human stratum corneum and the inert membrane (devoid of "shunts"), which is contrary to the simulation data (Figure 4.1), indicates that there is only a very small contribution by "shunt" transport to the overall permeation of steroids through the excised human stratum corneum (Figures 4.2 and 4.3). This is confirmed by diffusion model II which also predicted a very small contribution of "shunt" transport to the overall permeation of polar and non-polar steroids through excised human abdominal stratum corneum (Table 4.2). The permeability coefficients estimated by linear regression of the steady state data (Scheuplein et al 1969) and those estimated by non-linear regression using diffusion model I (Table 4.4) were qualitatively of similar magnitude, indicating that an adequate interpretation of the data could be made using a simple linear regression approach.

The present results have shown that the main route of skin penetration of steroids through excised human stratum corneum is more likely to be through the "intracellular" route than through the "shunts". However, specific reports by different workers (section 2.2.1) indicate that it will be very difficult to determine the relative importance of each route of percutaneous penetration for steroids, as in situ a substance absorbed through hair follicles may diffuse in the dermis and may reappear again due to back diffusion in the subepidermal layers.

CHAPTER 5

Permeation of weak electrolytes through the human stratum corneum

Knowledge of the mechanism by which drugs traverse the skin is important in developing strategies for the control of drug delivery through the skin. Although it is recognised that drugs may penetrate the skin via "shunts" and/or by the "intracellular" route, the relative contribution of each of these pathways to the overall transport of a drug through the skin is poorly understood (Chapters 1 and 2). The transport of weak electrolytes through the skin may be further complicated by the potential absorption of both ionised and unionised drug through "shunts" and/or the "intracellular" route. In order to examine the mechanism of drug transport through the skin, the percutaneous absorption of a model substance (methotrexate) has been studied under a variety of vehicle pH conditions. These studies were further validated by using other compounds.

Methotrexate was chosen as the model substance for this work as the possibility of "shunt" and "intracellular" transport of this substance has been previously suggested (Wallace and Barnett 1978). The possibility of drug/vehicle interactions (section 2.3.1.5) can be accounted for by the measurement of fluxes through an inert membrane with identical conditions (Roberts and Anderson 1975; Roberts and Horlock 1978). Differences between the fluxes of the solute through the inert membrane and skin reflect alteration in the physicochemical nature of the skin in the presence of the vehicle. As an inert membrane is devoid of "shunts", transport through the "shunts" in the skin may possibly

be deduced by a comparison of the rates of permeation through the skin and the inert membrane (Chapter 4).

This section describes studies in which the pH of the vehicle was varied and the penetration of methotrexate, and other weak electrolytes, through the skin and through an inert membrane followed with time. The major aim of this section of the work was to describe the pH dependence of methotrexate (and other weak electrolyte) penetration using an appropriate mathematical model.

Theory

Two classical models are usually used to describe the transport of solutes in biological systems and are referred to as the diffusion approach (section 2.5; Table 2.5) and the compartmental approach (Wallace and Barnett 1978; Table 2.6). The diffusion model (Chapter 4) is widely used in describing the transport of solutes through membranes and is characterized by a defined resistance barrier throughout the membrane (Crank 1975). In contrast, the compartmental model assumes that the resistance to the transport across the solution-skin-solution interfaces are the well stirred individual phases (solution,skin) in the system. This latter model has been used previously to describe the transport of methotrexate through hairless mouse skin (Wallace and Barnett 1978).

The permeation of a weak electrolyte through the stratum corneum can also be related to permeation through a simple membrane provided it is assumed that the total diffusional resistance of the skin is due only to the stratum corneum and also that the

physical characteristics of the stratum corneum are not altered by the application of the drug (Scheuplein 1978b; Chapter 4).

Diffusion equations

(1) Unionised solute transport through the stratum corneum: The cumulative amount of solute penetrating the membrane (M) in time (t) is given by:

$$M = C_u Kh \left\{ D/h^2 \, t - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp\left(-n^2 \pi^2 \, Dt/h^2\right) \right\}$$

Eq. 5.1

where C_u is the concentration of the unionised solute in the donor compartment, K is the solute-membrane partition coefficient and D is the diffusion coefficient of solute for the membrane with thickness h (Crank 1975). Equation 5.1 (Figure 5.1a) is frequently used to describe the transport of solutes through intact stratum corneum (Scheuplein 1978b). Equation 5.1 is subsequently referred to as diffusion model I or DM I.

Equation 5.1 can also be expressed in terms of the permeability coefficient (k_p) where:

$$k_{p_u} = \frac{KD}{h} = Kh \times D/h^2 \qquad \text{Eq. 5.2}$$

The slope of the steady state portion of the plot of M versus time in equation 5.1 corresponds to the steady state flux (J_{ss}) and the intercept of this plot on the time axis is referred to as the lag time (t_L). The lag time for diffusion model I is given by Crank (1975) as:

$$t_L = h^2/6D \qquad \text{Eq. 5.3}$$

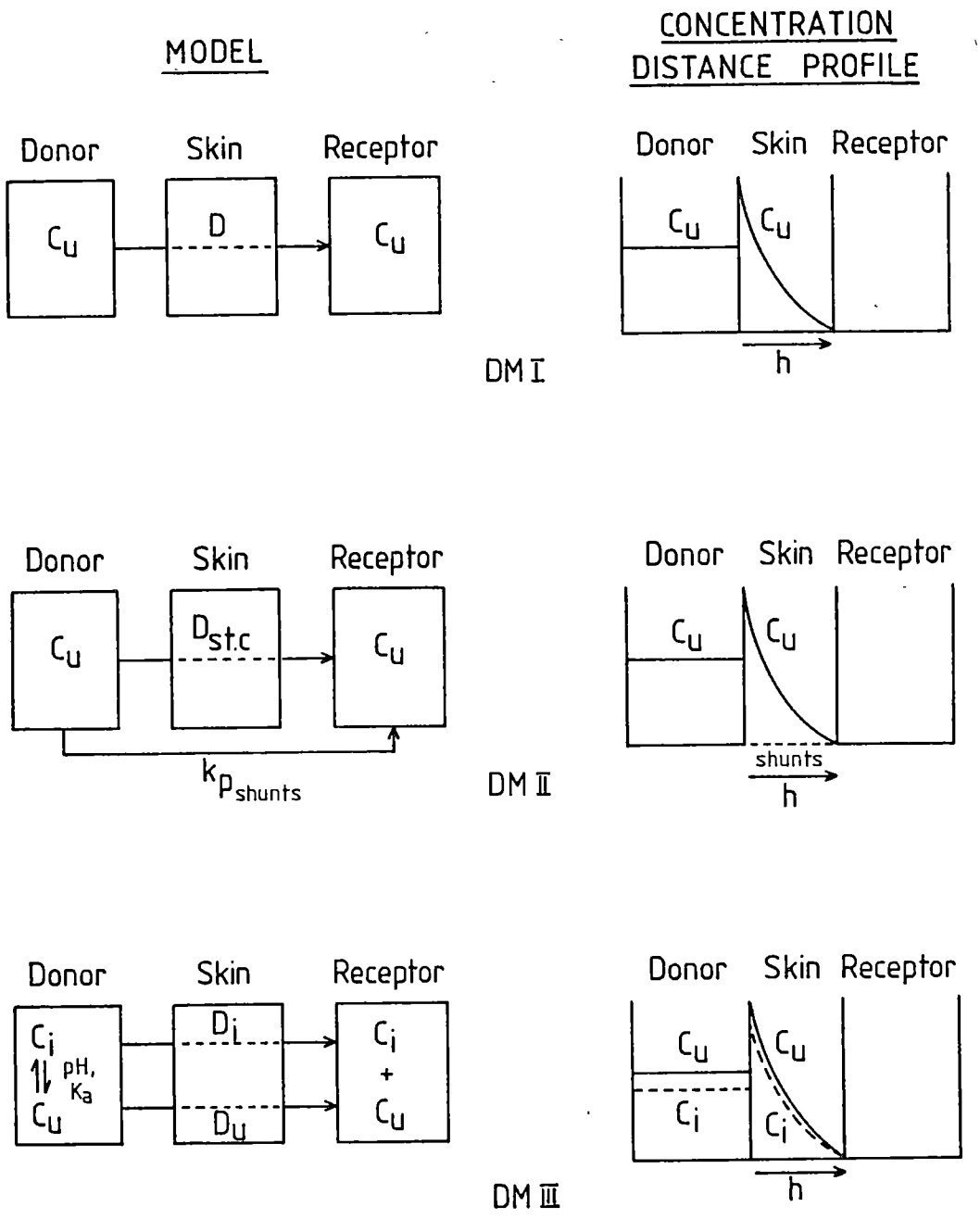


Figure 5.1(a) A comparison of diffusion models.

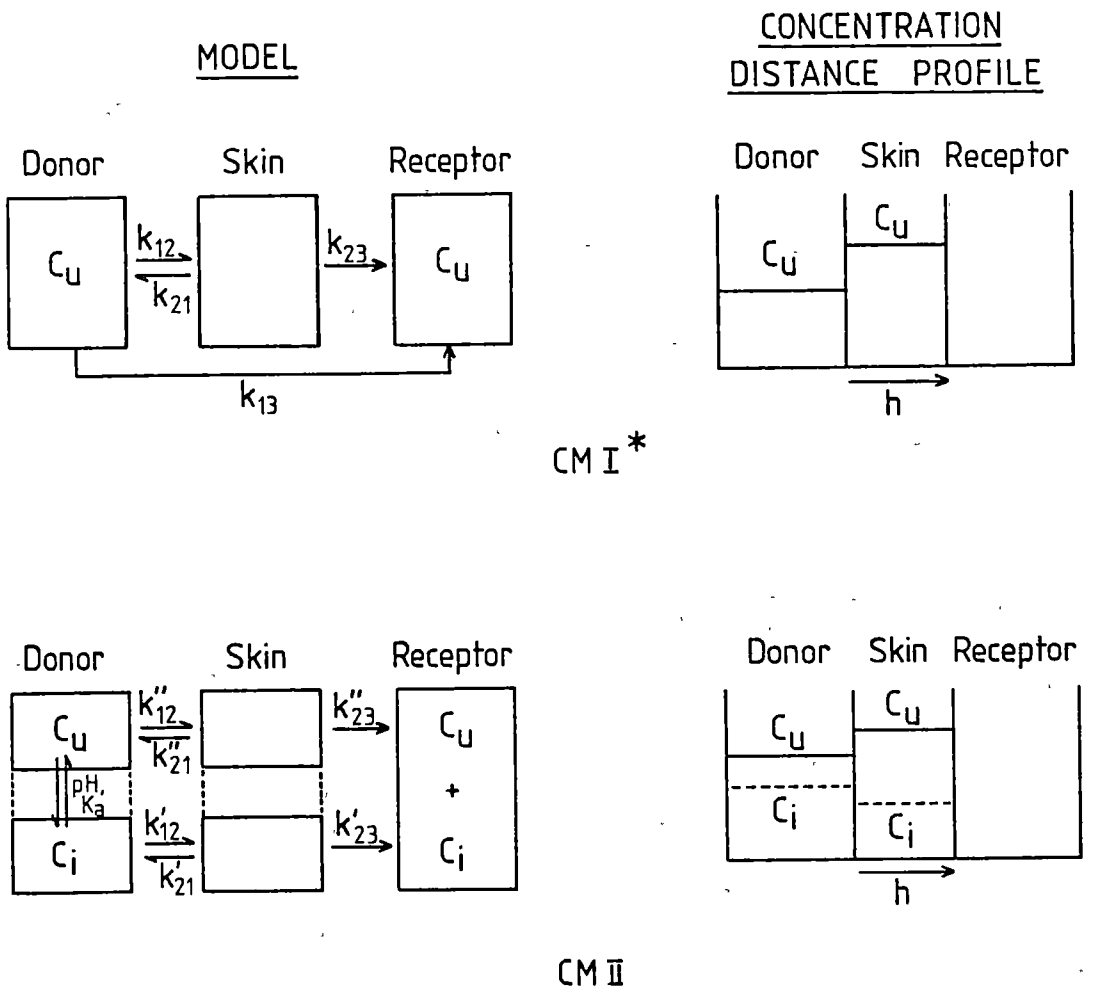


Figure 5.1(b) A comparison of compartment models.
(*Scheme from Wallace and Barnett 1978).

(2) Unionised solute transport through the shunts: Transport of solutes through "shunts" is characterized by large diffusion coefficients and small fluxes, the latter reflecting a small surface area occupied by the shunts (Scheuplein 1978b; Barry 1983; section 2.2.1). Hence, in this analysis the lag time for the transport of solute through "shunts" is assumed to be negligible. The cumulative amount of solute (M_s) penetrating the skin via "shunts" is therefore given by:

$$M_s = C_u k_{p_s} t \qquad \text{Eq. 5.4}$$

(3) Total unionised solute transport: If a solute penetrates the skin through both the intact stratum corenum ("intracellular" route) and the "shunts", the total amount transported is given by summing equations 5.1 and 5.4 (Figure 5.1a).

Consequently the cumulative amount of diffusant (M) per unit area that passes through the stratum corneum by both routes of skin penetration is given by:

$$M = \underbrace{C_u K_e h_e \left\{ D_e/h_e^2 - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp\left(n^2 \pi^2 Dt/h_e^2\right) \right\}}_{\text{"intracellular" route}} + \underbrace{C_u k_{p_s} t}_{\text{"shunts"}} \qquad \text{Eq. 5.5}$$

Equation 5.5 is subsequently referred to as diffusion model II or DM II.

As $t \to \infty$ equation 5.5 becomes,

$$M = \frac{C_u K_e D_e}{h_e} \left(t - h^2/6D_e\right) + C_u k_{p_s} t \qquad \text{Eq. 5.6}$$

The lag time is the intercept of the plot of the cumulative amount permeated against time on the time axis of equation 5.5.

Therefore, solving for equation 5.5 for M = 0, one can obtain the lag time for diffusion model II as:

$$t_L = \frac{K_e h_e}{6k_{p_e} + 6k_{p_s}} \qquad \text{Eq. 5.7}$$

(4) Unionised and ionised solute transport: The amount of weak electrolytes penetrating the intact stratum corneum is assumed to be the sum of the unionised (u) and ionised (i) solute transport with the cumulative amount of diffusant (M) per unit area passing through the skin being given by:

"unionised" transport

$$M = C_u K_u h_u \left\{ D_u/h_u^2 t - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp\left(-n^2\pi^2 D_u t/h_u\right) \right\}$$

"ionised" transport

$$+ C_i K_i h_i \left\{ D_i/h_i^2 t - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \exp\left(-n^2\pi^2 D_i t/h_i\right) \right\}$$

Eq. 5.8

Equation 5.8 is subsequently referred to as diffusion model III or DM III (Figure 5.1a).

Equation 5.8 approximates to equation 5.9 as $t \to \infty$.

$$M = \frac{C_u K_u D_u}{h_u}\left(t - h_u^2/6D_u\right) + \frac{C_i K_i D_i}{h_i}\left(t - h_i^2/6D_i\right) \qquad \text{Eq. 5.9}$$

The slope of the plot of M versus time corresponds to the steady

state flux (J_{ss}) and the x-axis intercept corresponds to the lag time (t_L) i.e.

$$J_{ss} = C_u k_{p_u} + C_i k_{p_i} \qquad \text{Eq. 5.10}$$

and

$$t_L = \frac{C_u K_u h_u + C_i K_i h_i}{6(C_u k_{p_u} + C_i k_{p_i})} \qquad \text{Eq. 5.11}$$

Compartmental equations

(1) Total unionised solute transport through the stratum corneum: A model based on compartmental pharmacokinetics (CM I, Figure 5.1b) has been reported by Wallace and Barnett (1978) and expressed mathematically as:

$$M = C_u k^u_{12} t - \frac{C_u k^u_{12}}{k^u_{23}} \left(1 - \exp^{-k^u_{23} t}\right) \qquad \text{Eq. 5.12}$$

where C_u = unionised donor concentration and k_{21}, k_{23} are intercompartmental rate constants (Figure 5.1b). Equation 5.12 is subsequently referred to as compartmental model I or CM I (Appendix 1).

(2) Unionised and ionised solute transport: The transport of unionised and ionised solute through the human skin can be described by compartmental analysis using the model described in Figure 5.1b (CM II). The equations for this model are derived in Appendix 2. The final equation, assuming constant input is:

"ionised" transport

$$M = C_i k^i_{12} t - \frac{C_i k^i_{12}}{k^i_{23}} \left(1 - \exp^{-k^i_{23} t}\right)$$

"unionised" transport

$$+ C_u k^u_{12} t - \frac{C_u k^u_{12}}{k^u_{23}} \left(1 - \exp^{-k^u_{23} t}\right) \qquad \text{Eq. 5.13}$$

Equation 5.13 is subsequently referred to as compartmental model II or CM II.

As $t \rightarrow \infty$, equation 5.13 can also be written as:

$$M = \left(C_i k_{12}^i + C_u k_{12}^u\right) \times \left\{t - \left(\frac{C_i k_{12}^u}{k_{23}^i} + \frac{C_u k_{12}^u}{k_{23}^u}\right) \middle| \left(C_i k_{12}^i + C_u k_{12}^u\right)\right. \qquad \text{Eq. 5.14}$$

In equation 5.14 the lag time and steady state flux (slope of M against t) can be represented by the following set of equations:

$$t_L = \left(\frac{C_i k_{12}^i}{k_{23}^i} + \frac{C_u k_{12}^u}{k_{23}^u}\right) \middle| \left(C_i k_{12}^i + C_u k_{12}^u\right) \qquad \text{Eq. 5.15}$$

and

$$J_{ss} = C_i k_{12}^i + C_u k_{12}^u \qquad \text{Eq. 5.16}$$

5.1 Experimental

Permeation studies of nine weak electrolytes (Table 3.1) were carried out using the steady state permeation cell (Figure 3.1) and the method described in sections 3.3.1 and 3.4. Partition coefficients with and without radioactive counter ion, (K' and K''), were measured using the method described in section 3.11. Individual data representing the cumulative amount of solute recovered in the receptor compartment against time for various pH values for the permeation of each of a number of weak

electrolytes through human stratum corneum and the inert membrane (silastic) were fitted simultaneously (unweigted data of all pH runs) using a non-linear regression programme and equations derived from diffusion and compartmental modelling (section 3.7.5). The models used are given by equations 5.1 (DM I), 5.5 (DM II), 5.8 (DM III), 5.12 (CM I) and 5.13 (CM II). Steady state flux and lag times (linear regression) were also calculated by the method described in section 2.5 (Figure 2.3).

Permeation studies (sections 3.3.1 and 3.4) were also carried out using radioactive counter ion (rubidium or chloride) with and without the weak electrolytes at three pH values (high, medium and low degree of ionisation). The permeability coefficient (section 2.5) was then calculated from the number of counts recovered in the receptor compartment and the counts remaining in the donor compartment for each substance.

The fraction of maximum value of partition coefficient was calculated by dividing the partition coefficient at each pH value by the maximum unionised partition coefficient obtained for the weak electrolyte i.e. $K_{max} = K'/fu$.

5.2 Results

5.2.1 Methotrexate

Simple permeation through stratum corneum

Figure 5.2 shows the data points of the cumulative amount of methotrexate in the receptor compartment with time for each of the two different initial concentrations. The figure shows that

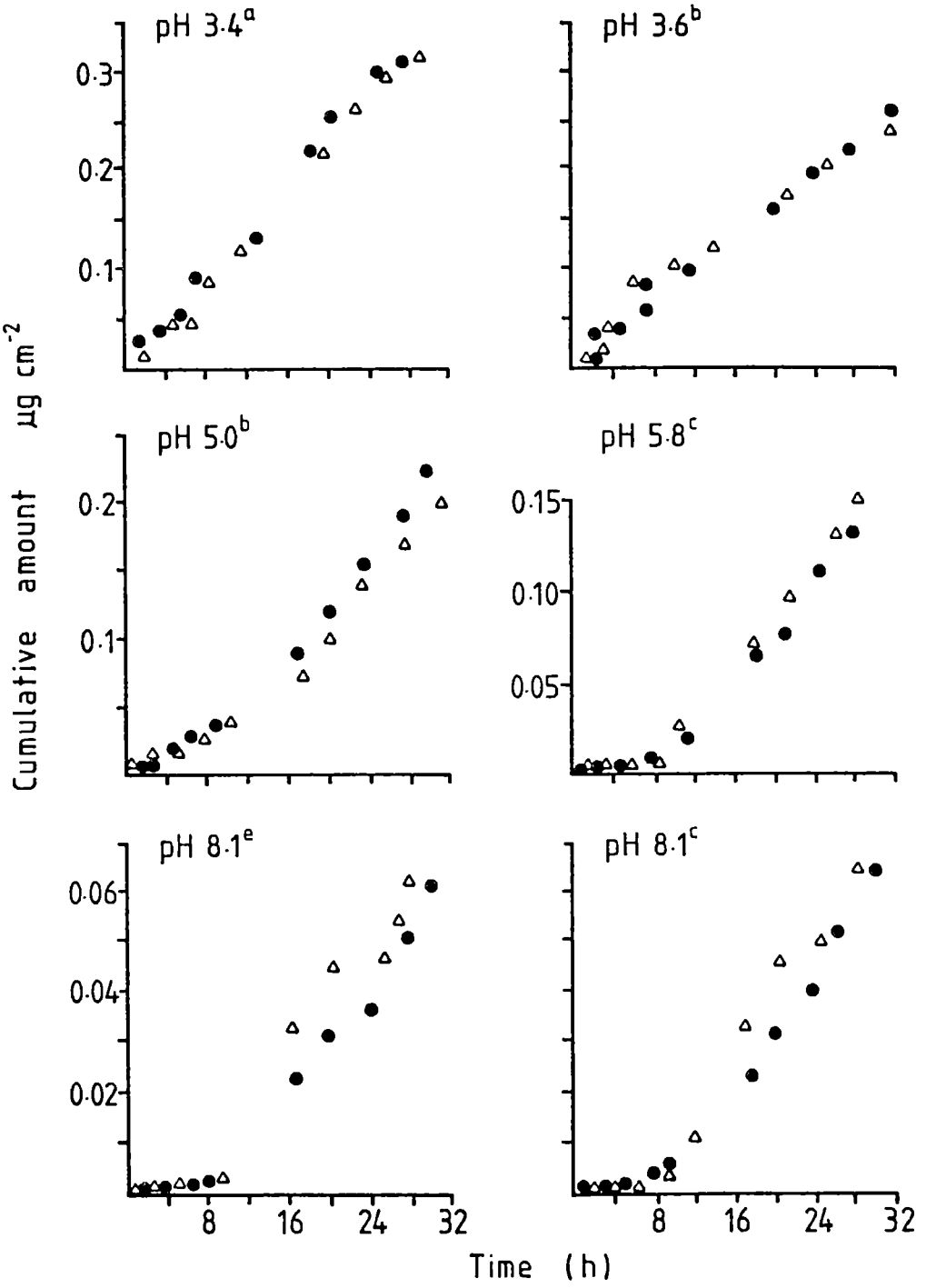


Figure 5.2 Permeation of methotrexate through the human stratum corneum. a,b,c and e are for different buffers shown in table 3.2

the rate of permeation is independent of concentration. The coefficient of variation between the values of the cumulative amount penetrated after 30 hours at pH 8.1 was found to be about 1% (N = 3). An analysis of variance of the data in Figure 5.2 shows that the steady state flux and the lag times were pH dependent ($F = 1032$, $df = 4/10$, $P < 0.05$).

Mathematical models - regression

Figures 5.3 and 5.4 shows the predicted cumulative amount of methotrexate penetrating at each pH value using compartment model I and diffusion model I and the non-linear regression of the data. The cumulative amounts permeated at the higher pH's are poorly described by the regression lines obtained using diffusion model I and compartment model I indicating that these models are inappropriate for the description of transport of methotrexate through the human stratum corneum.

Figures 5.3 and 5.4 also shows that the permeation of methotrexate is dependent on both the ionised and unionised concentrations and that the data can be adequately described at all pH's by compartmental model II and diffusion model III. The models used in this evaluation assumes that both the unionised and ionised species of methotrexate diffuse through the skin. Residual analysis shows that the data points are randomly distributed. The amounts permeated, at all pH's, also show a random scatter around the regression lines of CM II and DM III. These regressions were obtained by simultaneous fitting of all the data.

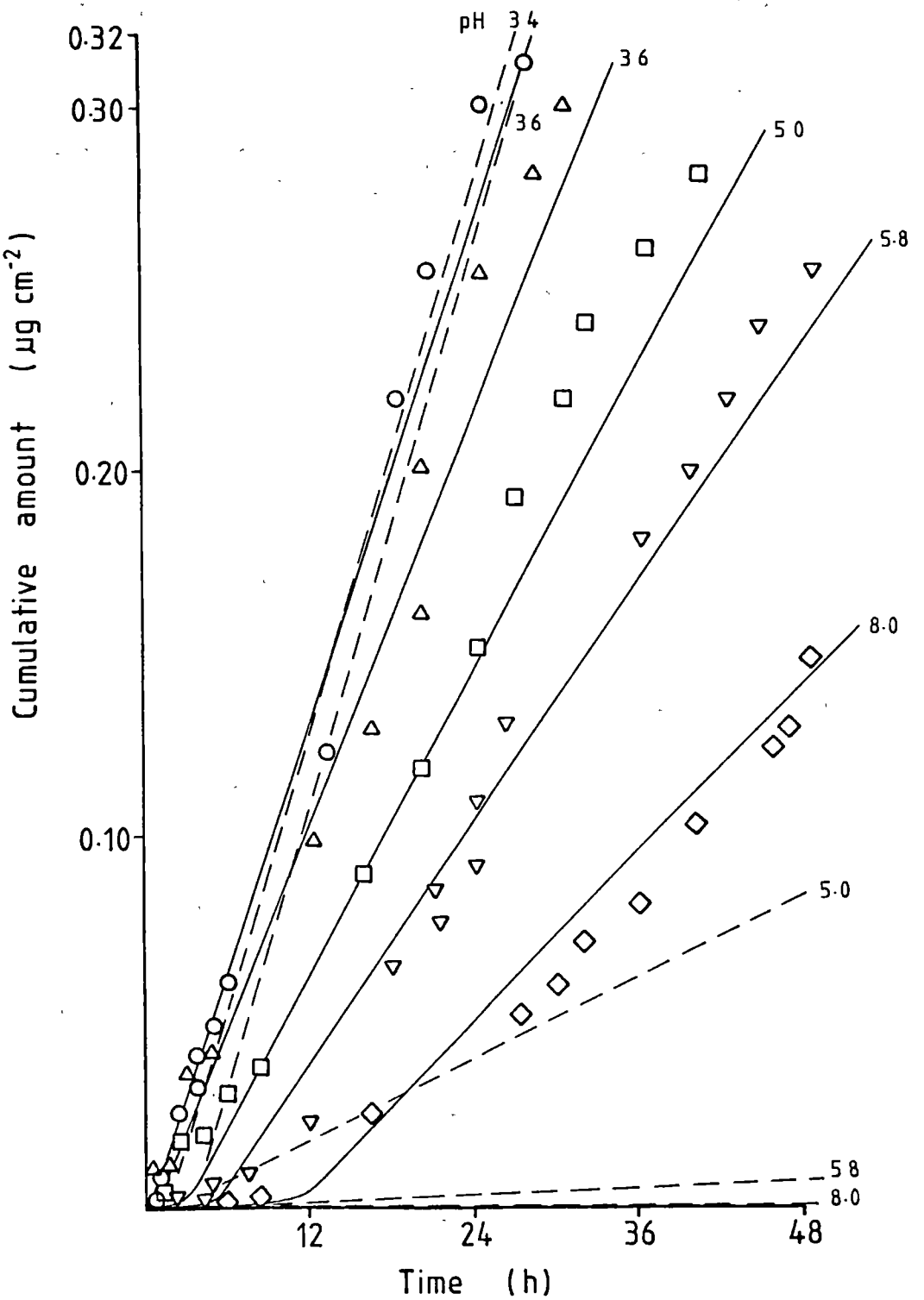


Figure 5.3 Permeation of methotrexate through the human stratum corneum using buffer e (table 3.2). The solid and broken lines are the results predicted by CM II and CM I respectively. ○ pH 3.4, △ pH 3.6, □ pH 5.0, ▽ pH 5.8, ◇ pH 8.0.

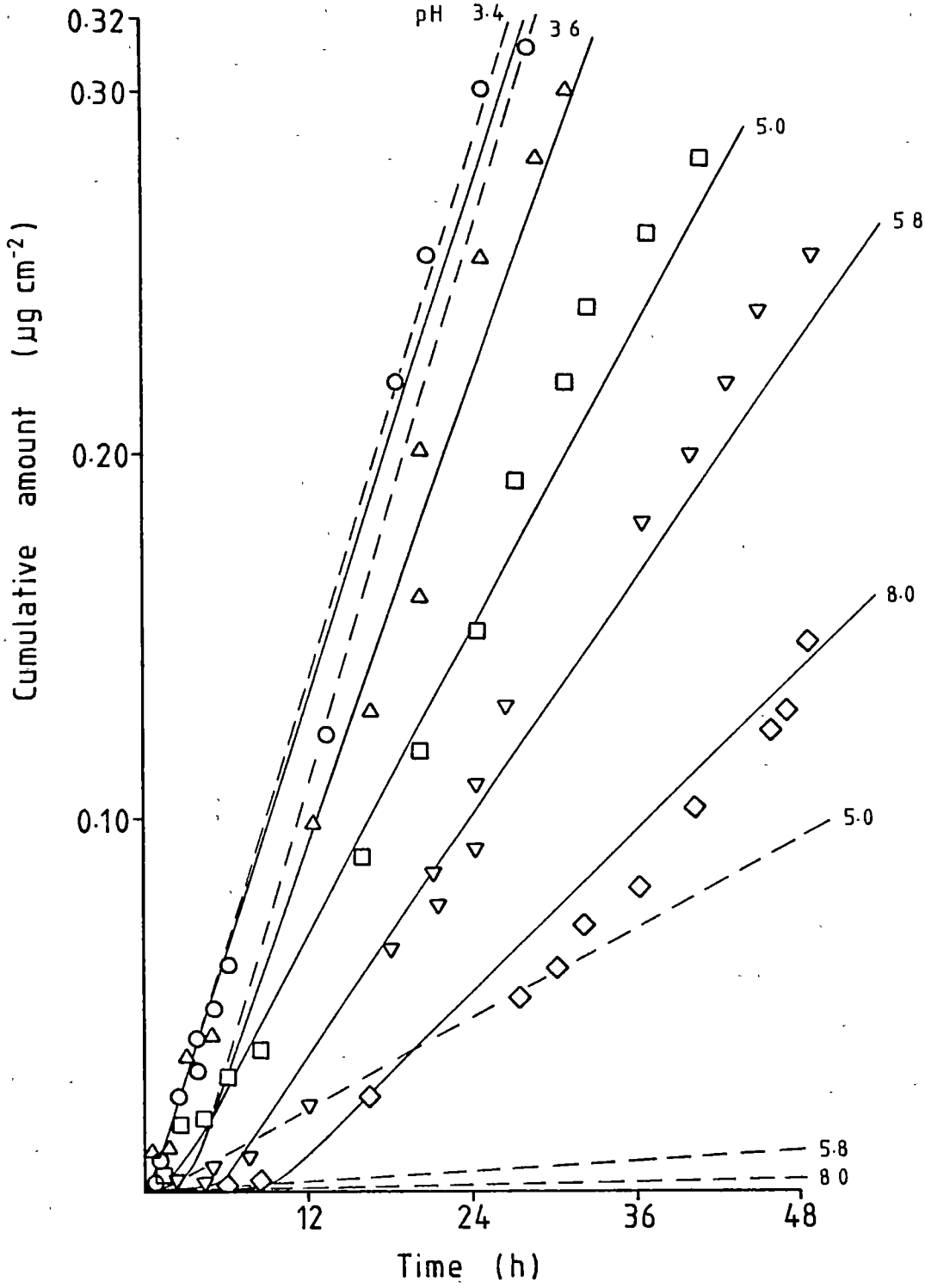


Figure 5.4 Permeation of methotrexate through the human stratum corneum using buffer e (table 3.2). The solid and broken lines are the results predicted by DM III and DM I respectively. ○ pH 3.4, △ pH 3.6, □ pH 5.0, ▽ pH 5.8, ◇ pH 8.1.

Diffusion model I and compartmental model I assumes that only the unionised species of the drug is able to permeate the human skin. The amounts permeating at higher pH's were poorly described by the regression lines obtained, indicating that both these models are inappropriate for the description of the transport of methotrexate through human stratum corneum.

Figures 5.3 and 5.4 also show that the amount of methotrexate permeated is a consequence of the passage of both the ionised and the unionised species of the drug. It is also apparent that the data is adequately described by diffusion model III and compartmental model II provided that the ionised and unionised donor concentrations are taken into account. Residual analysis shows that the data points are randomly distributed. The amounts penetrating at all pH's show a random scatter around the regression lines (Figures 5.3 and 5.4).

Comparison of models

Table 5.1 shows the parameter estimates for the diffusion models I, II and III. When the parameters (k_p) in diffusion models I and II are compared with similar parameter in diffusion model III, a high coefficient of variation (%CV) is obtained for diffusion models I and II. A similar trend in %CV is found for the lag time and exponential coefficient ($k_{21} + k_{23}$) in compartmental model I (Table 5.2).

An analysis of variance was carried out to compare the residual sum of squares differences between the fits for the models (Boxenbaum et al 1974). A significant difference was found

Table 5.1 Final parameter values for the permeation of methotrexate through the human stratum corneum using diffusion models (N = 72).

Parameter	Diffusion models (DM)		
	DM I	DM II	DM III
$k_{p_u} \times 10^4$	5.00 (34)	-	3.86 (10)
$k_{p_e} \times 10^3$	-	8.20 (65)	-
$k_{p_s} \times 10^4$	-	3.00 (32)	-
$k_{p_i} \times 10^4$	-	-	1.54 (12)
t_L	1.99 (320)	66.88 (2)	-
t_{L_u}	-	-	0.24 (368)
t_{L_i}	-	-	4.27 (80)
% response explained	36.67	40.67	80.97

The units for k_p and t_L are cm/hr and hrs respectively.
The numbers in parenthesis are % CV's.

Table 5.2 Final parameter values for the permeation of methotrexate through the human stratum corneum using compartmental models (N = 72).

Parameter	Compartmental models (CM)	
	CM I	CM II
$k_p \times 10^4$	4.67 (19)	-
t_L	1.63 (192)	-
$k_{21} + k_{23}$	0.18 (516)	-
k^i_{23}	-	0.25 (89)
$k^i_{12} \times 10^4$	-	1.52 (13)
k^u_{23}	-	0.96 (3)
$k^u_{12} \times 10^4$	-	4.00 (6)
% response explained	37.48	82.30

The units of k_p, t_L and k are cm/hr, hrs and hr^{-1} respectively. The numbers in parenthesis are % CV.

between diffusion model I and diffusion model III (Appendix 3), and also between diffusion model II and diffusion model III ($F = 160$, $df = 1/68$). A significant difference was also found between compartmental model I and compartmental model II (Appendix 3).

Steady state flux and lag times

Table 5.3 shows the steady state flux and lag times estimated using DM III, CM II and linear regression. Flux predicted by the models are of similar magnitude to those estimated by linear regression approach. However, it was not possible to correctly estimate the lag times using DM III or CM II.

Figure 5.5 shows that the steady state flux for the stratum corneum and the inert membrane (linear regression) was also found to be related to the pH (i.e. degree of ionisation) of the aqueous solution. As the extent of ionisation increases the flux through the inert membrane decreases. However, the magnitude of the reduction in the flux for the inert membrane with the increase in the extent of ionisation of methotrexate in aqueous solutions, was very small in comparison to the flux obtained for the permeation of methotrexate through the human stratum corneum (Figure 5.5). The lag times for the inert membrane were found to show a very small change with pH relative to that seen for permeation through the human stratum corneum.

Partition coefficient

The apparent octanol-buffer partition coefficient for total concentration (unionised + ionised) of methotrexate (K') is related to pH (Figure 5.5). As the extent of ionisation increases

Table 5.3 Steady state flux and lag times for the permeation of methotrexate through the human stratum corneum using DM III*, CM II* and linear regression (LR).

pH (25°C)	f_u	J_{ss} (ng cm^{-2} hr^{-1})			Lag times (hrs)		
		DM III	CM II	LR	DM III	CM II	LR
3.4[e]	0.887	10.8	11.2	13.3	0.4	1.1	0.8
3.6[e]	0.832	10.4	10.8	11.1	0.4	1.2	1.6
5.0[e]	0.132	5.5	5.6	7.0	2.8	2.9	4.3
5.8[e]	0.011	4.7	4.7	5.6	4.2	3.9	10.0
8.0[e]	< 0.001	4.0	4.6	3.8	4.3	4.0	11.3

*Calculated from the final parameter values shown in Tables 5.1 (DM III) and 5.2 (CM II). e is the type of buffer shown in Table 3.2.

f_u is the fraction unionised.

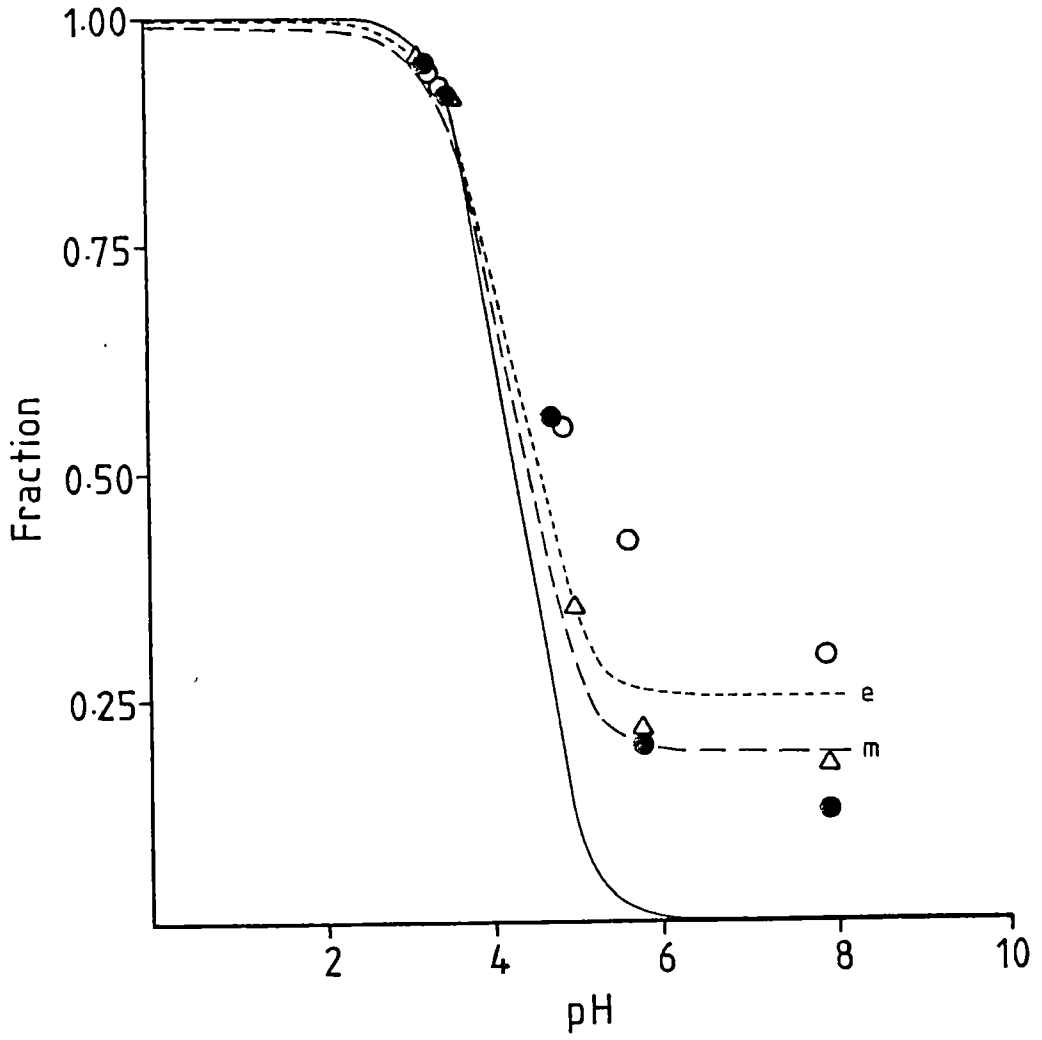


Figure 5.5 Effect of vehicle pH on the steady state flux (J_{ss}) and partition coefficient for methotrexate. The solid line was predicted by DM I. The broken lines were predicted by DM III for the stratum corneum (e) and the inert membrane (m). ○ J_e-LR; △ J_m-LR and ● K.

there is a decrease in the partition coefficient; K' was also found to vary with the type of buffer system used as a vehicle (Table 5.4). On the other hand the apparent octanol-water partition coefficient (K'') for the methotrexate-rubidium complex was found to increase with ionisation (Figure 5.6); K'' for rubidium only was found to be independent of ionisation. It was also found that the K'' values of the methotrexate complex were dependent upon the concentration of methotrexate present in the system.

5.2.2 Compounds other than methotrexate

Simple permeation through the stratum corneum

The coefficient of variation between the values of the cumulative amount of weak electrolytes recovered in the receptor compartment, after 4 to 6 hours at a pH where the weak electrolyte was almost totally unionised, was found to be around 2-3.5% (n = 3) for each of the compounds. An analysis of variance of the raw data in Figures 5.7 to 5.13 shows that the steady state flux and the lag times are pH dependent (Appendix 4).

Permeation studies were also carried out for approximately 24-30 hours for all the other compounds at a pH value which gave a high degree of ionisation. The steady state flux and lag times obtained for the permeation of weak electrolytes for more than 24 hours were found to be of similar magnitude to those estimated for the original studies carried out for 5-6 hours (section 3.4).

Table 5.4 Effect of buffer constituents on apparent octanol/buffer partition coefficient (K') for weak electrolytes

pH	Methotrexate x 10^3	Salicylic acid	Aspirin	Lignocaine hydrochloride	Chlorpromazine hydrochloride	Chlorpheniramine maleate x 10^3	Ephedrine hydrochloride x 10^2
3.4[a]	51 ± 10.0	-	4.00 ± 0.20	1.2 ± 0.13	-	50 ± 10	-
3.6[b]	45 ± 9.0	4.60 ± 1.7	3.70 ± 0.12	-	8.0 ± 1.0	-	23 ± 1
5.0[a]	30 ± 10.0	3.70 ± 0.4	1.20 ± 0.25	-	-	-	-
5.0[b]	28 ± 9.0	3.30 ± 0.1	1.10 ± 0.20	-	-	-	-
5.2[e]	-	-	0.98 ± 0.21	1.7 ± 0.20	9.0 ± 0.5	80 ± 10	-
5.8[c]	12 ± 3.0	-	-	-	-	-	-
5.8[d]	11 ± 2.0	-	-	-	-	-	-
6.6[e]	-	0.50 ± 0.10	0.42 ± 0.04	-	-	-	31 ± 4
7.2[e]	-	0.47 ± 0.08	0.41 ± 0.10	1.8 ± 0.08	14.7 ± 0.5	140 ± 15	37 ± 5
8.1[c]	5 ± 1.0	0.38 ± 0.04	0.39 ± 0.05	2.0 ± 0.07	-	-	43 ± 12
8.1[d]	5 ± 1.5	0.41 ± 0.06	0.40 ± 0.04	2.2 ± 0.08	-	-	47 ± 13
8.1[e]	6 ± 0.8	0.42 ± 0.08	0.40 ± 0.05	2.6 ± 0.10	-	-	46 ± 10
8.4[f]	-	-	-	-	16.7 ± 0.4	190 ± 40	-
9.4[f]	-	-	-	3.0 ± 0.12	19.4 ± 0.6	470 ± 70	-
9.6[f]	-	-	-	-	-	-	55 ± 11
11.7[f]	-	-	-	3.2 ± 0.11	23.0 ± 1.0	750 ± 100	80 ± 29
3.6 (Tetra)	40 ± 9.0	1.70 ± 0.10	0.70 ± 0.21	1.6 ± 0.10	6.8 ± 0.4	330 ± 50	34 ± 7
5.2 (Penta)	20 ± 6.0	1.00 ± 0.3	0.07 ± 0.20	2.7 ± 0.22	9.8 ± 0.3	550 ± 20	40 ± 6

± SD, n = 4.

a to f are for the buffer systems shown in Table 3.2.

Tetra - 0.06M Tetrabutylammonium hydrogen sulphate (MW = 340) in water.

Penta - 0.06M 1 - Pentane sulfonic acid sodium salt monohydrate (MW = 192) in water.

*pH measured at 25°C.

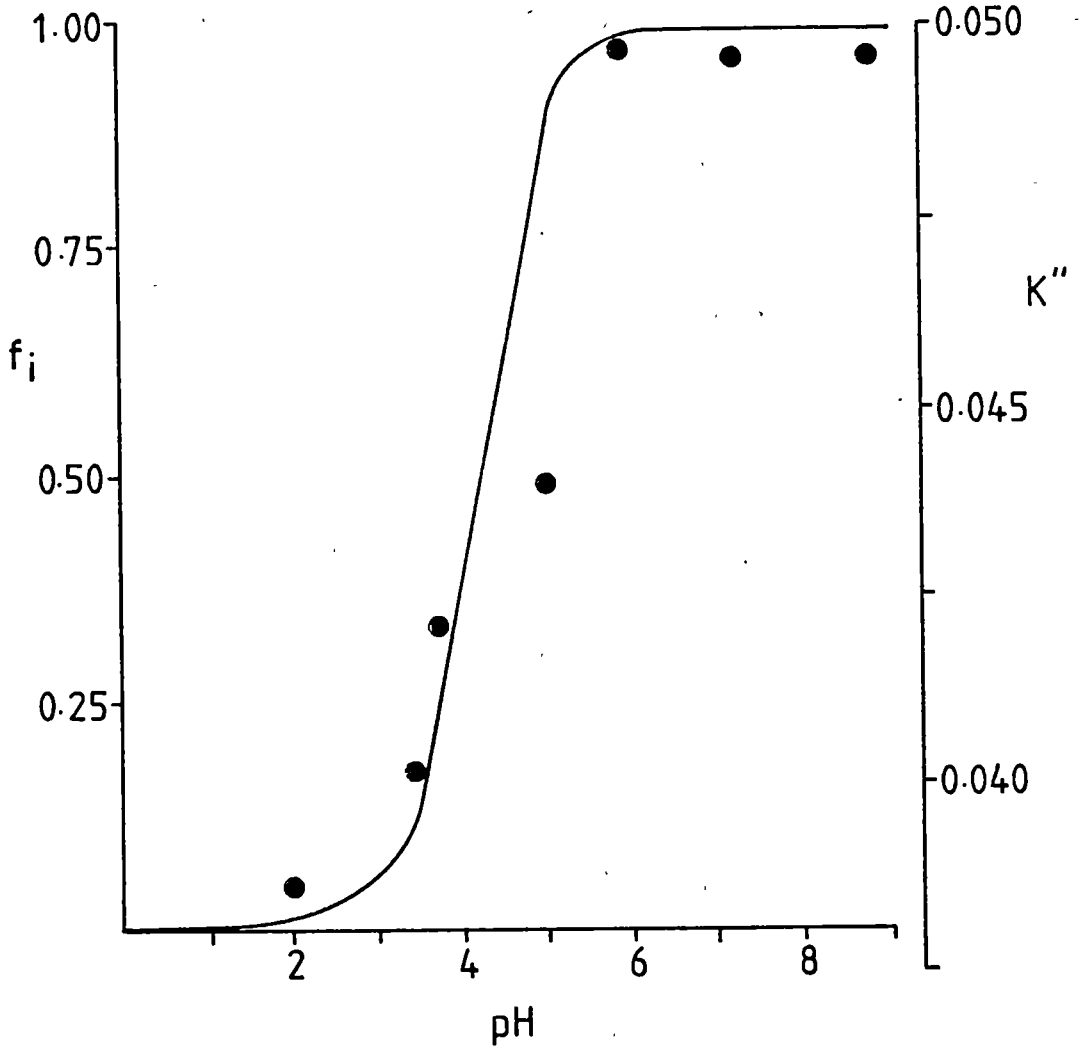


Figure 5.6 Relationship between partition coefficient (K'' ●) and degree of ionisation of methotrexate in aqueous solution (solid line).

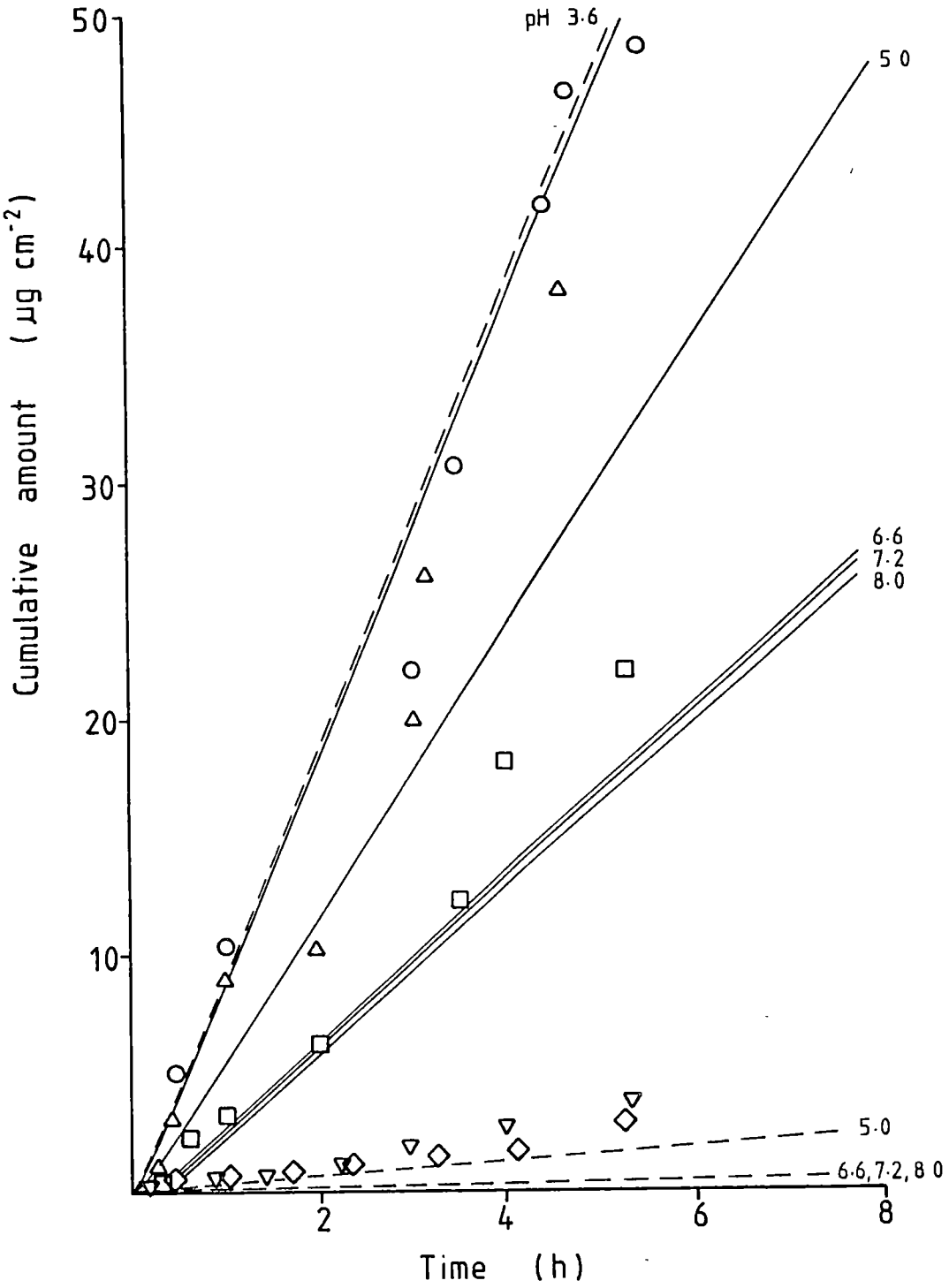


Figure 5.7 Permeation of salicylic acid through human stratum corneum. The solid and broken lines are the result predicted by DM III and DM I respectively. ○ pH 3.6, △ pH 5.0, □ pH 6.6, ▽ pH 7.2, ◇ pH 8.0.

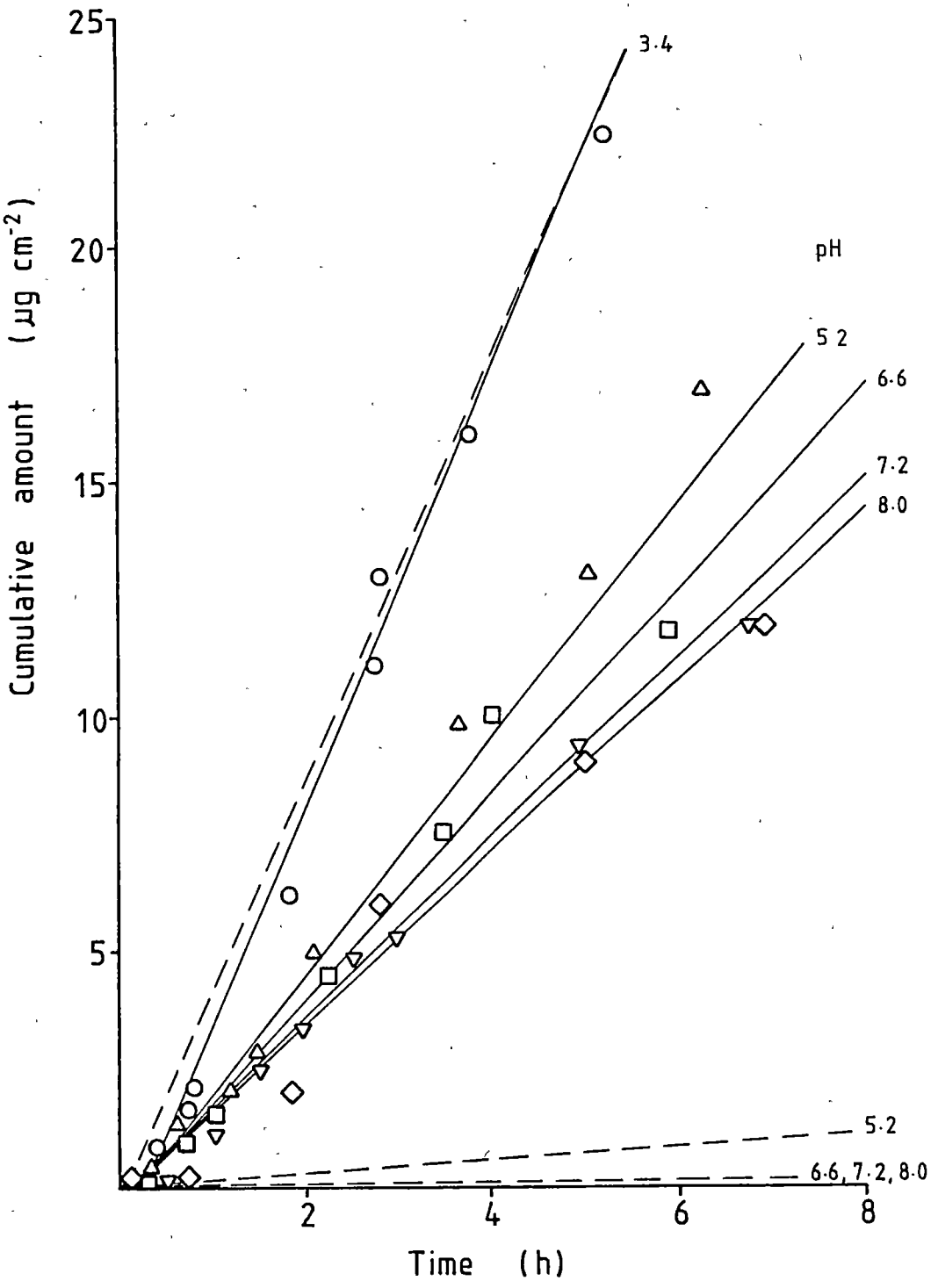


Figure 5.8 Permeation of aspirin through the human stratum corneum. The solid and broken lines are the results predicted by DM III and DM I respectively. ○ pH 3.4, △ pH 5.2, □ pH 6.6, ▽ pH 7.2, ◇ pH 8.0.

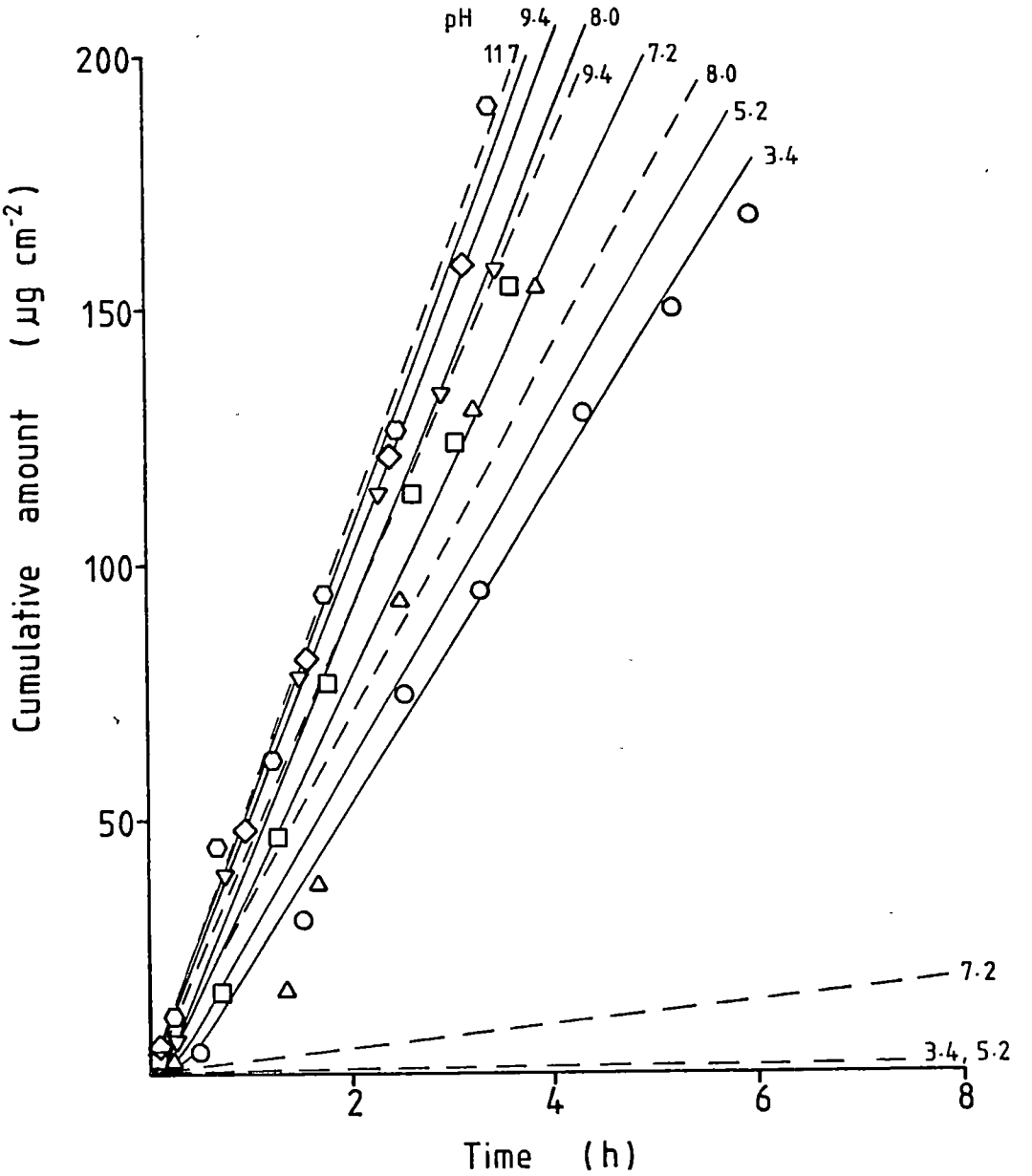


Figure 5.9 Permeation of lignocaine hydrochloride through the human stratum corneum. The solid and broken lines are the results predicted by DM III and DM I respectively. ○ pH 3.4, △ pH 5.2, □ pH 7.2, ▽ pH 8.0, ◇ pH 9.4, ⬡ pH 11.7.

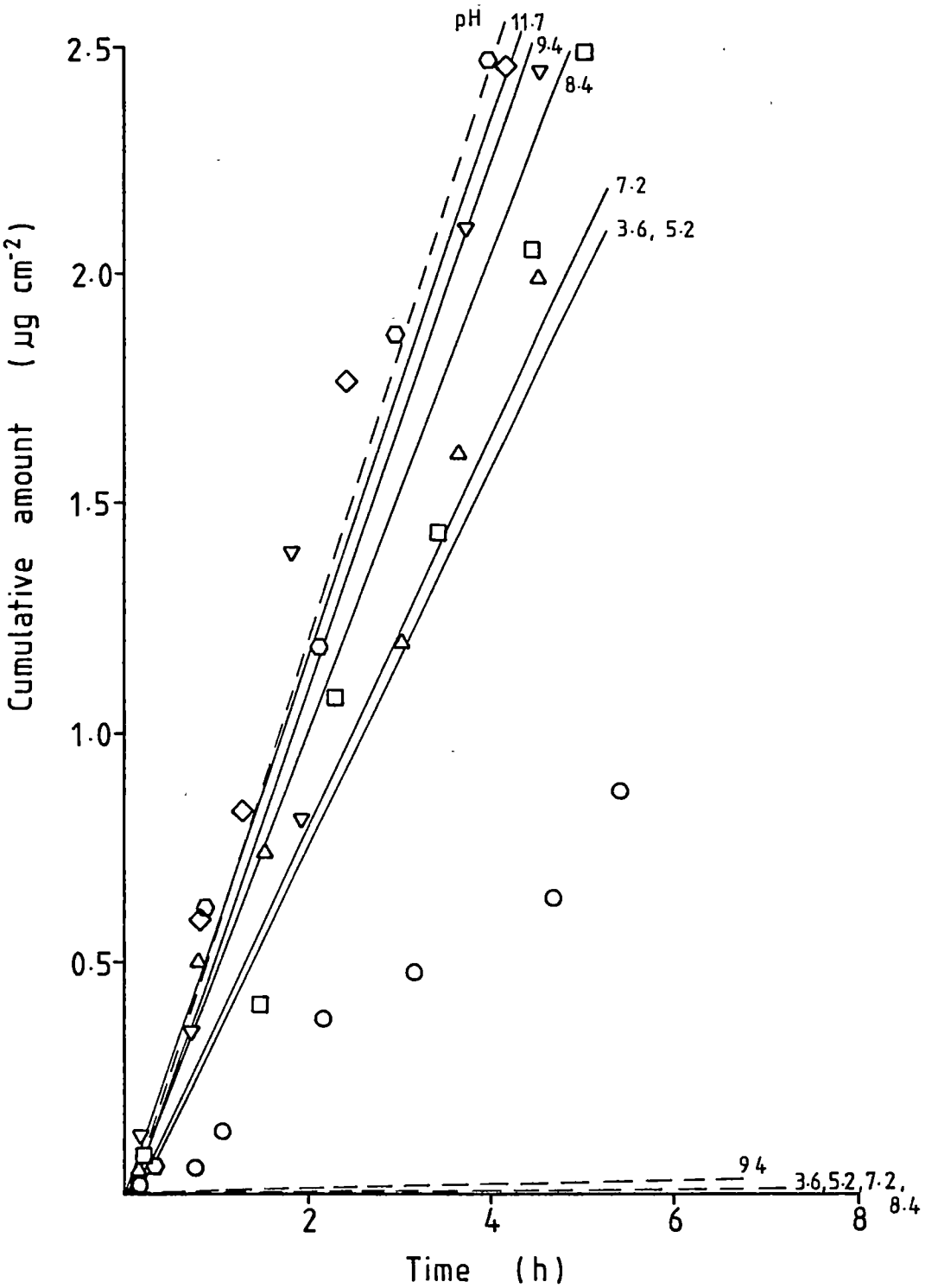


Figure 5.10 Permeation of chlorpromazine hydrochloride through the human stratum corneum. The solid and broken lines are the results predicted by DM III and DM I respectively. ○ pH 3.6, △ pH 5.2, □ pH 7.2, ▽ pH 8.4, ◇ pH 9.4, ⬡ pH 11.7.

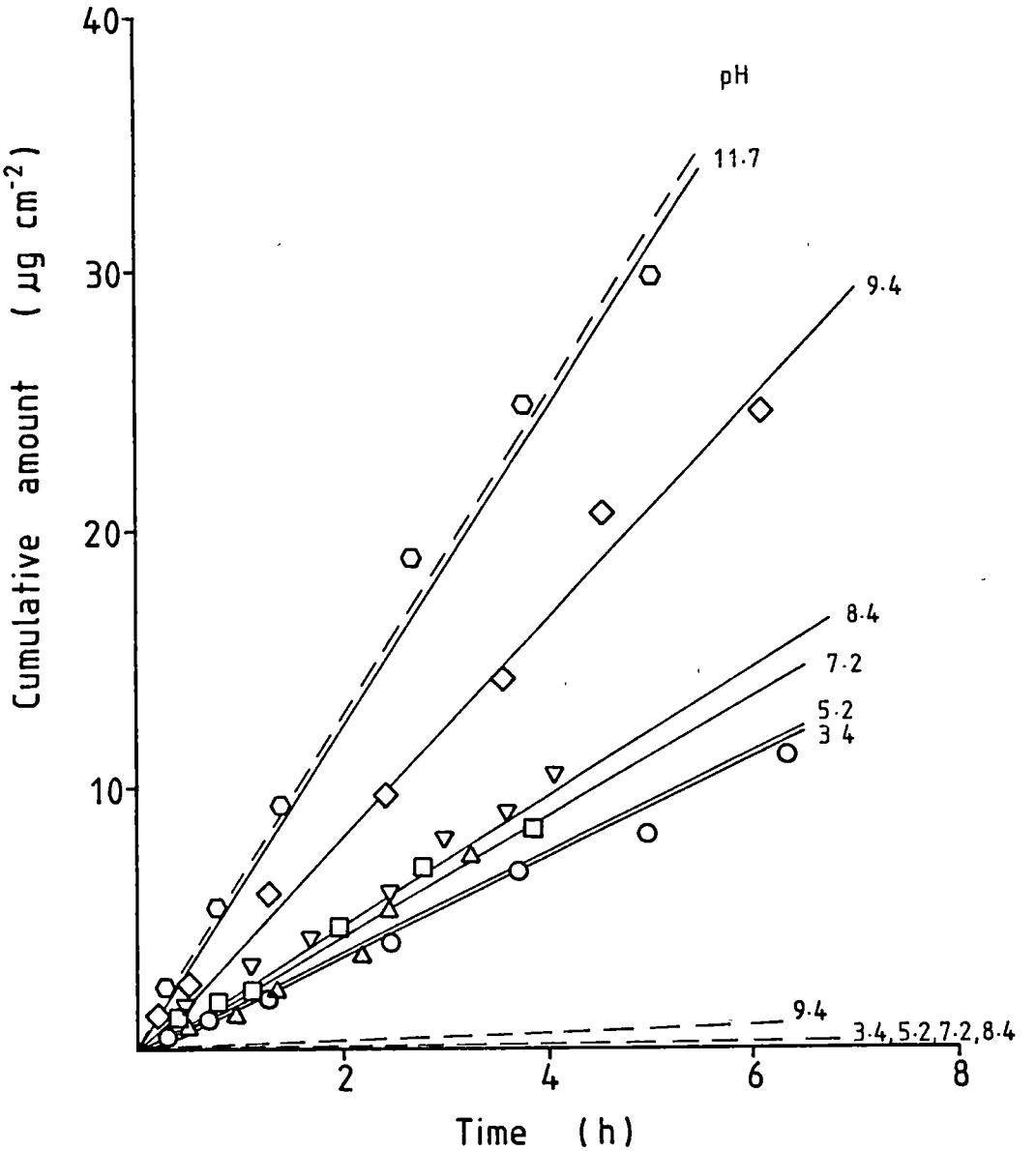


Figure 5.11 Permeation of chlorpheniramine maleate through the human stratum corneum. The solid and broken lines are the results predicted by DM III and DM I respectively. ○ pH 3.4, △ pH 5.2, □ pH 7.2, ▽ pH 8.4, ◇ pH 9.4, ⬡ pH 11.7.

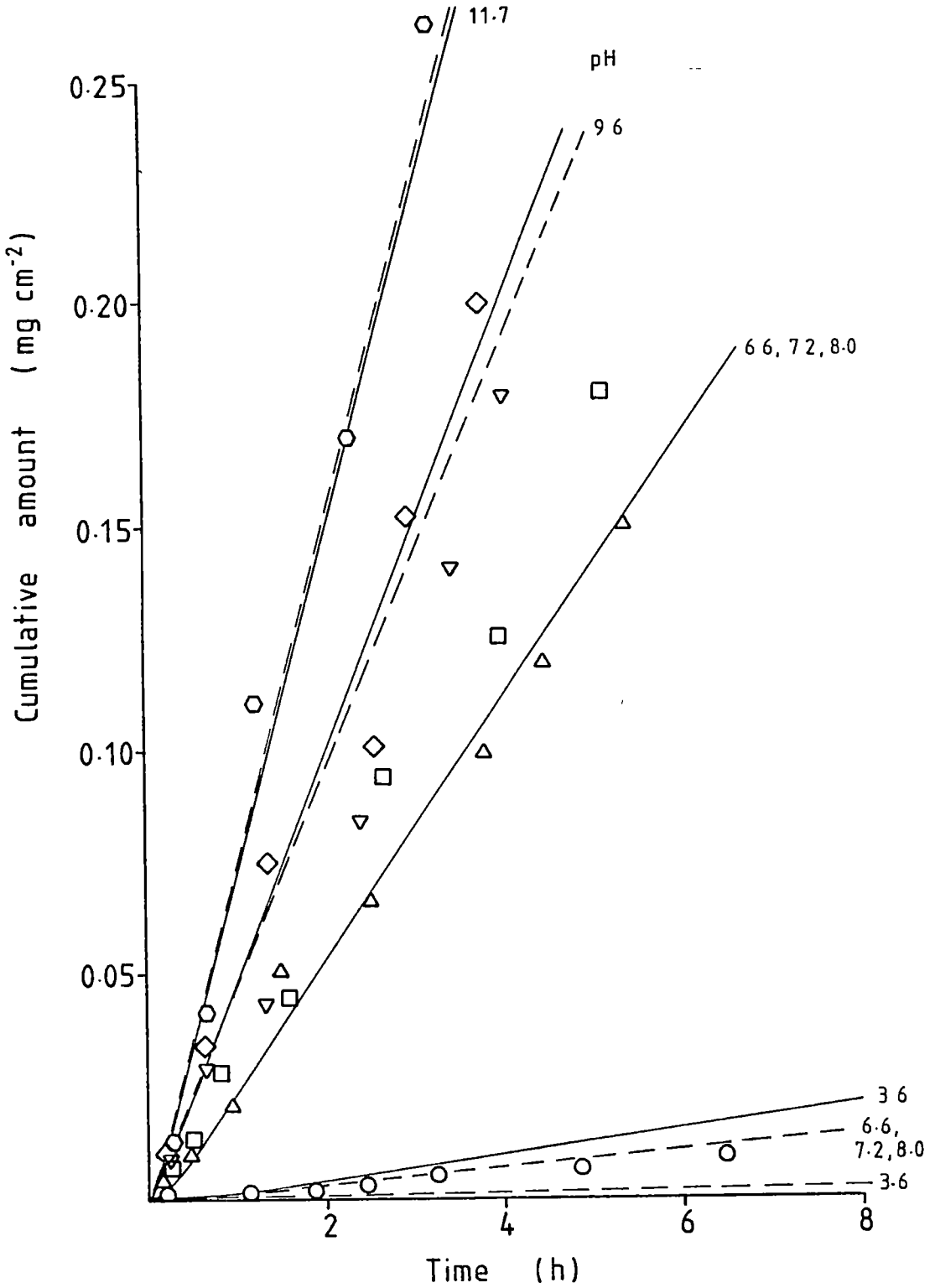


Figure 5.12 Permeation of ephedrine hydrochloride through the human stratum corneum. The solid and broken lines are the results predicted by DM III and DM I respectively.
○ pH 3.6, △ pH 6.6, □ pH 7.2, ▽ pH 8.0, ◇ pH 9.6, ⬡ pH 11.7.

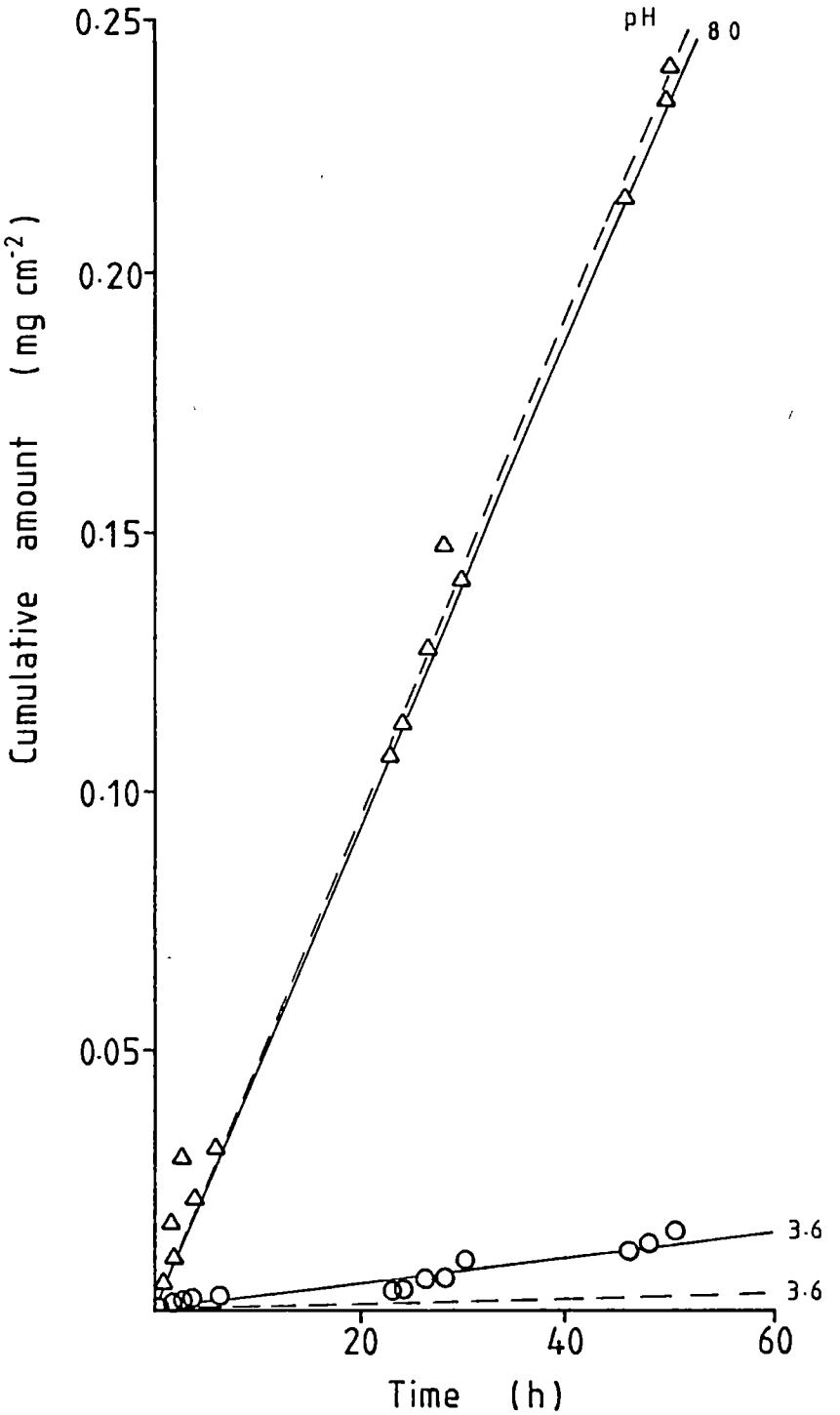


Figure 5.13 Permeation of pilocarpine hydrochloride through the human stratum corneum. The solid and broken lines are the results predicted by DM III and DM I respectively. ○ pH 3.6, △ pH 8.0.

Diffusion model III regression

Figures 5.7 to 5.13 show amounts predicted by diffusion model III. This model was found to adequately describe the in vitro permeation of all the compounds. Residual analysis of the regression fits (DM III) showed that the data points are randomly distributed. The amounts penetrating at all pH's also show a random scatter around the regression lines of diffusion model III for each compound (Figures 5.7 to 5.13).

Comparison of diffusion models

Table 5.5 shows the final parameter values for the diffusion models I and III. The %CV for the diffusion model III was generally found to be lower than those for the other model (Table 5.5). The F-test (Appendix 3) indicates the superiority of diffusion model III relative to the other diffusion model (DM I). Table 5.6 shows the final parameter estimates for the permeation of weak electrolytes through the inert membrane using diffusion model III. This model (DM III) was also able to adequately describe the transport of weak electrolytes through the inert membrane.

Steady state flux

Figures 5.14 to 5.19 show that the steady state flux for the human stratum corneum and the inert membrane (linear regression) was also found to vary with pH and the degree of ionisation of weak electrolytes in aqueous solutions. As the extent of ionisation increased the flux through the human stratum corneum and the inert membrane also decreased. The steady state flux for

Table 5.5 Final parameter values for the permeation of weak electrolytes using diffusion models.

Models - Parameters		Salicylic acid N = 29	Aspirin N = 30	Lignocaine hydrochloride N = 36	Chlorpromazine hydrochloride N = 35	Chlorpheniramine maleate N = 38	Ephedrine hydrochloride N = 36	Pilocarpine hydrochloride N = 26
DM I	$k_{p_u} \times 10^2$	5.50 (24)	1.80 (51)	7.00 (37)	0.75 (21)	1.00 (10)	14.20 (27)	8.20 (22)
	t_L	< 0.1	0.58 (270)	0.25 (283)	< 0.1	0.02 (1477)	0.16 (359)	< 0.1
% response explained		73.63	40.36	33.67	23.08	89.90	44.12	90.28
DM III	$k_{p_u} \times 10^2$	4.40 (25)	1.50 (12)	5.40 (8)	0.65 (9)	0.99 (2)	12.26 (14)	7.50 (4)
	$k_{p_i} \times 10^2$	0.30 (30)	0.43 (6)	3.50 (7)	0.57 (14)	0.33 (4)	4.10 (9)	0.37 (60)
	t_{L_u}	0.15 (482)	0.61 (56)	< 0.1	< 0.1	0.15 (50)	< 0.1	< 0.1
	t_{L_i}	< 0.1	0.15 (150)	0.14 (127)	< 0.1	< 0.1	< 0.1	< 0.1
% response explained		79.59	95.47	93.64	79.17	99.11	79.03	96.80

The units of k_p and t_L are cm/hr and hrs respectively.

The numbers in the parenthesis are % CV's.

Table 5.6 Parameter estimate of weak electrolytes (inert membrane) for diffusion model III

Parameter	k_{p_u} x 10^2	k_{p_i} x 10^2	t_{L_u}	t_{L_i}	% response explained
Methotrexate (N = 34)	2.80 (17)	0.53 (9)	1.5 (30)	< 0.1	86.96
Salicylic acid (N = 24)	4.30 (28)	0.46 (30)	< 0.1	< 0.1	81.02
Aspirin (N = 25)	1.50 (8)	0.63 (11)	< 0.1	0.14 (201)	92.17
Lignocaine hydrochloride (N = 28)	6.60 (8)	3.80 (7)	< 0.1	0.12 (145)	97.28
Chlorpromazine hydrochloride (N = 20)	1.30 (8)	5.00 (17)	< 0.1	< 0.1	87.06
Chlorpheniramine maleate (N = 22)	1.40 (7)	5.40 (16)	< 0.1	< 0.1	95.53
Ephedrine hydrochloride (N = 20)	14.10 (14)	42.00 (21)	< 0.1	< 0.1	84.34

The numbers in the parenthesis are the % CV.

The units of k_p and t_L are cm/hr and hrs respectively.

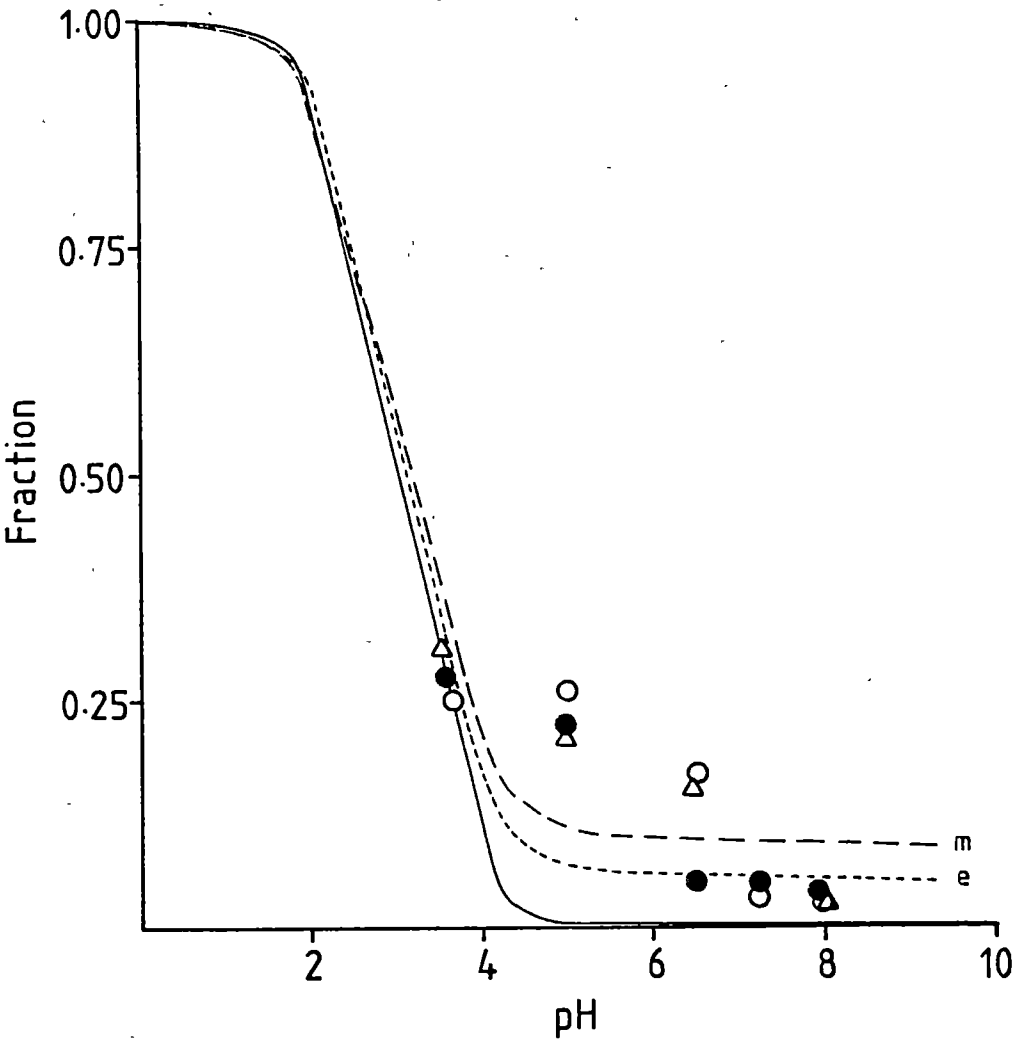


Figure 5.14 Effect of vehicle pH on the steady state flux (J_{ss}) and partition coefficient for salicylic acid. The solid line was predicted by DM I. The broken lines were predicted by DM III for the stratum corneum (e) and the inert membrane (m). ○ Je-LR; △ J_m-LR and ● K.

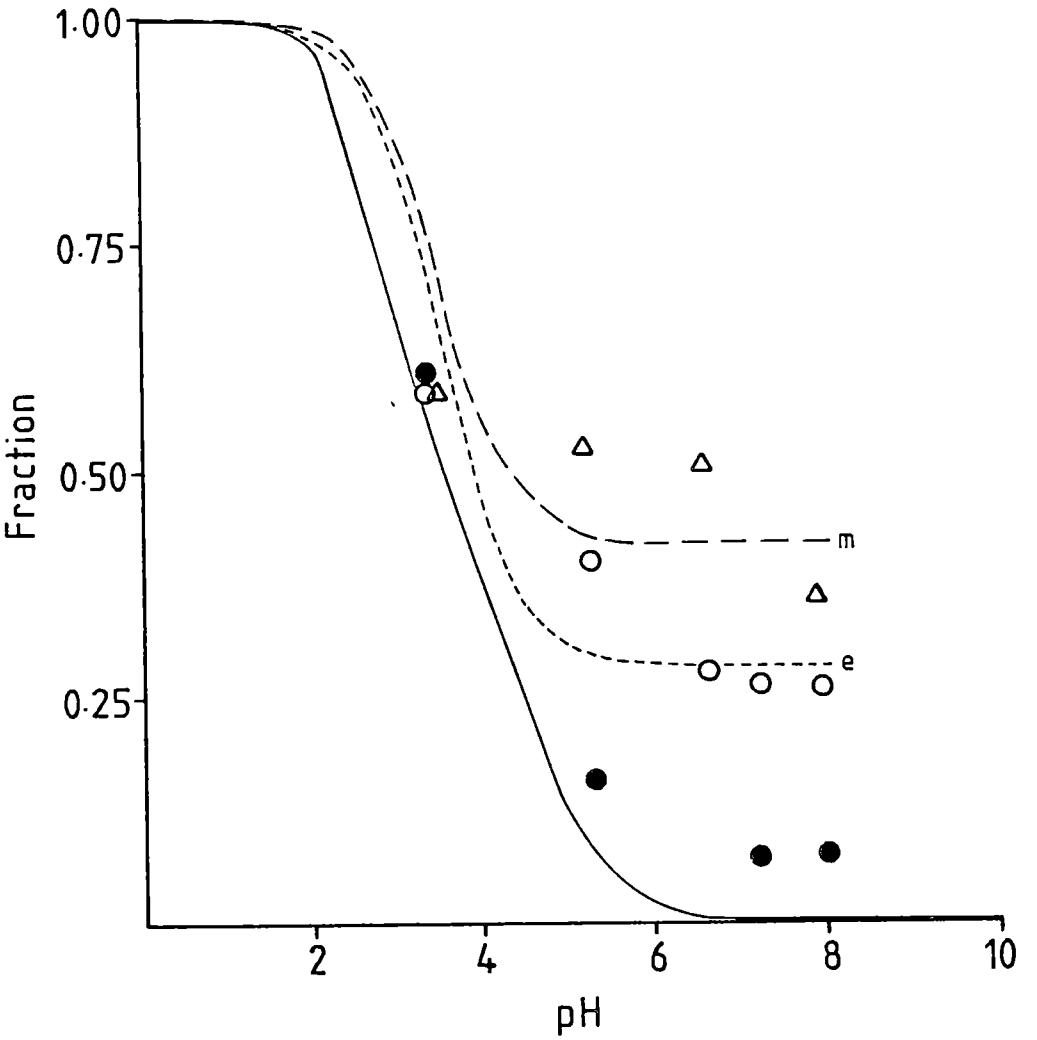


Figure 5.15 Effect of vehicle pH on the steady state flux (J_{ss}) and partition coefficient for aspirin. The solid line was predicted by DM I. The broken lines were predicted by DM III for the stratum corneum (e) and the inert membrane (m). ○ J_e-LR; △ J_m-LR and ● K.

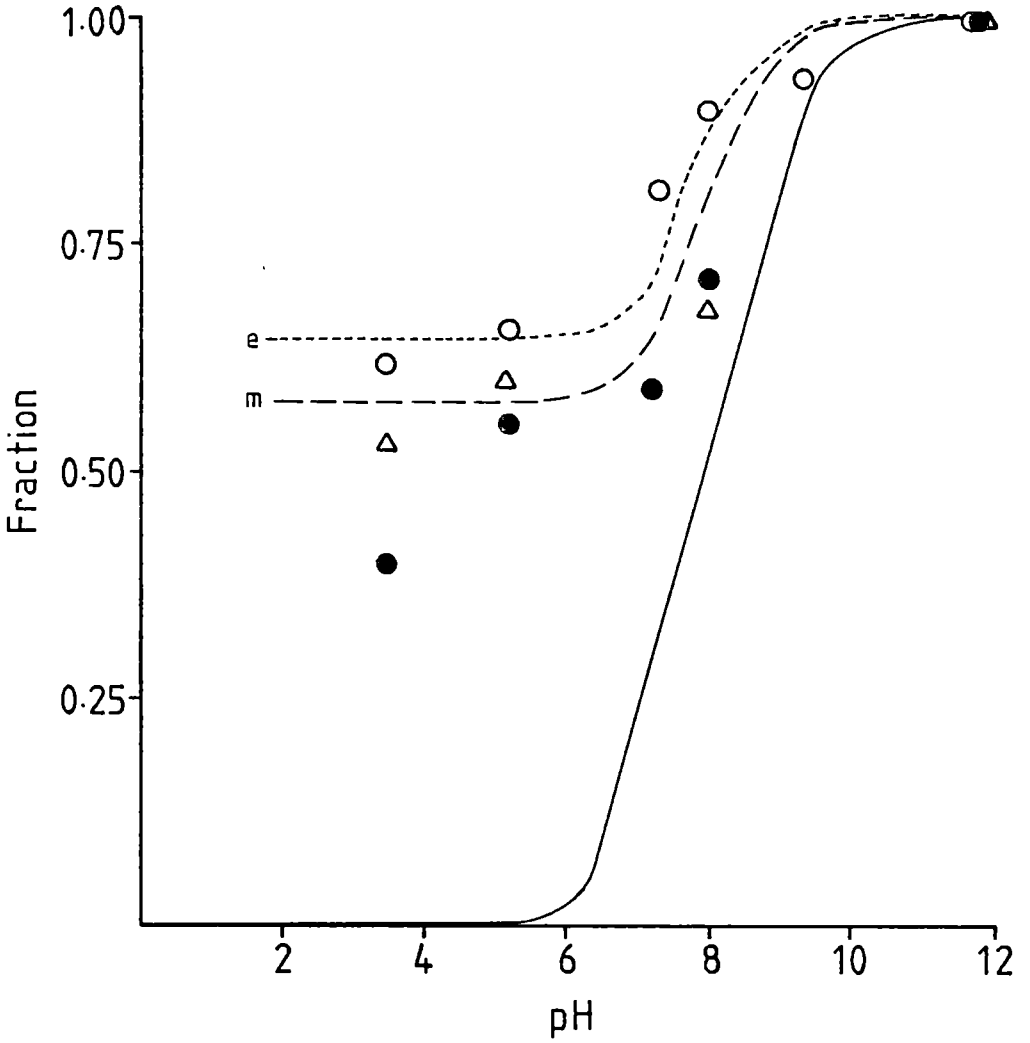


Figure 5.16 Effect of vehicle pH on the steady state flux (J_{ss}) and partition coefficient for lignocaine hydrochloride. The solid line was predicted by DM I. The broken lines were predicted by DM III for the stratum corneum (e) and the inert membrane (m). ○ J_e-LR; △ J_m-LR and ● K.

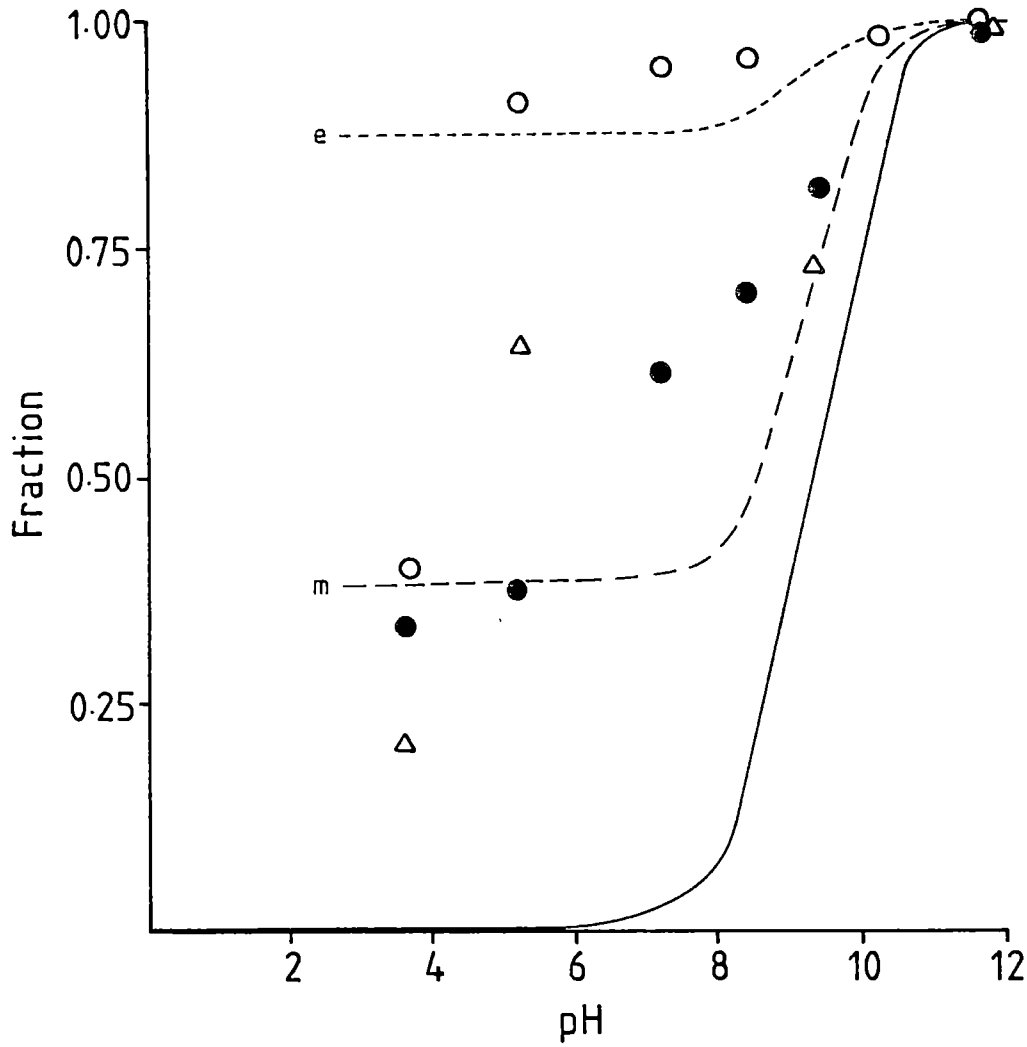


Figure 5.17 Effect of vehicle pH on the steady state flux (J_{ss}) and partition coefficient for chlorpromazine hydrochloride. The solid line was predicted by DM I. The broken lines were predicted by DM III for the stratum corneum (e) and the inert membrane (m). ○ J_e-LR; △ J_m-LR and ● K.

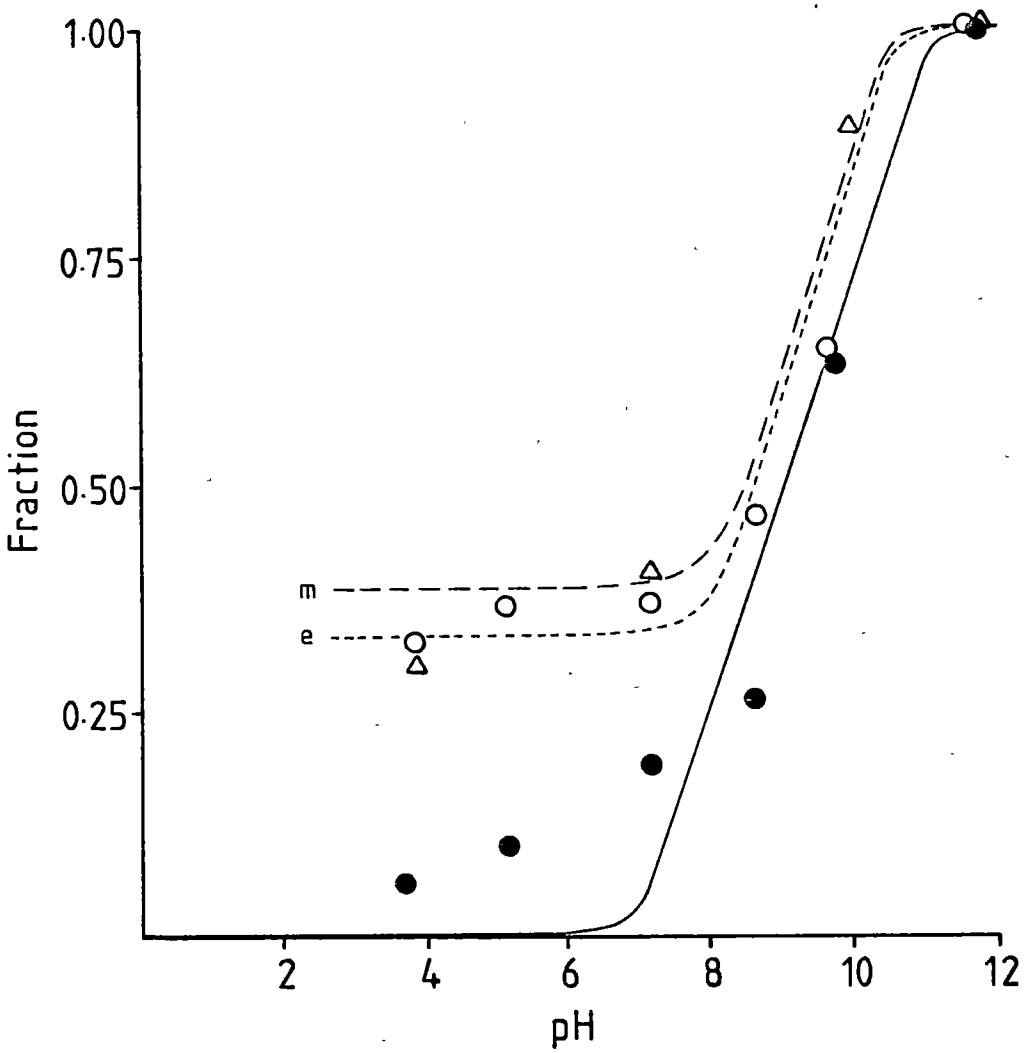


Figure 5.18 Effect of vehicle pH on the steady state flux (J_{ss}) and partition coefficient for chlorpheniramine maleate. The solid line was predicted by DM I. The broken lines were predicted by DM III for the stratum corneum (e) and the inert membrane (m). ○ J_e-LR; △ J_m-LR and ● K.

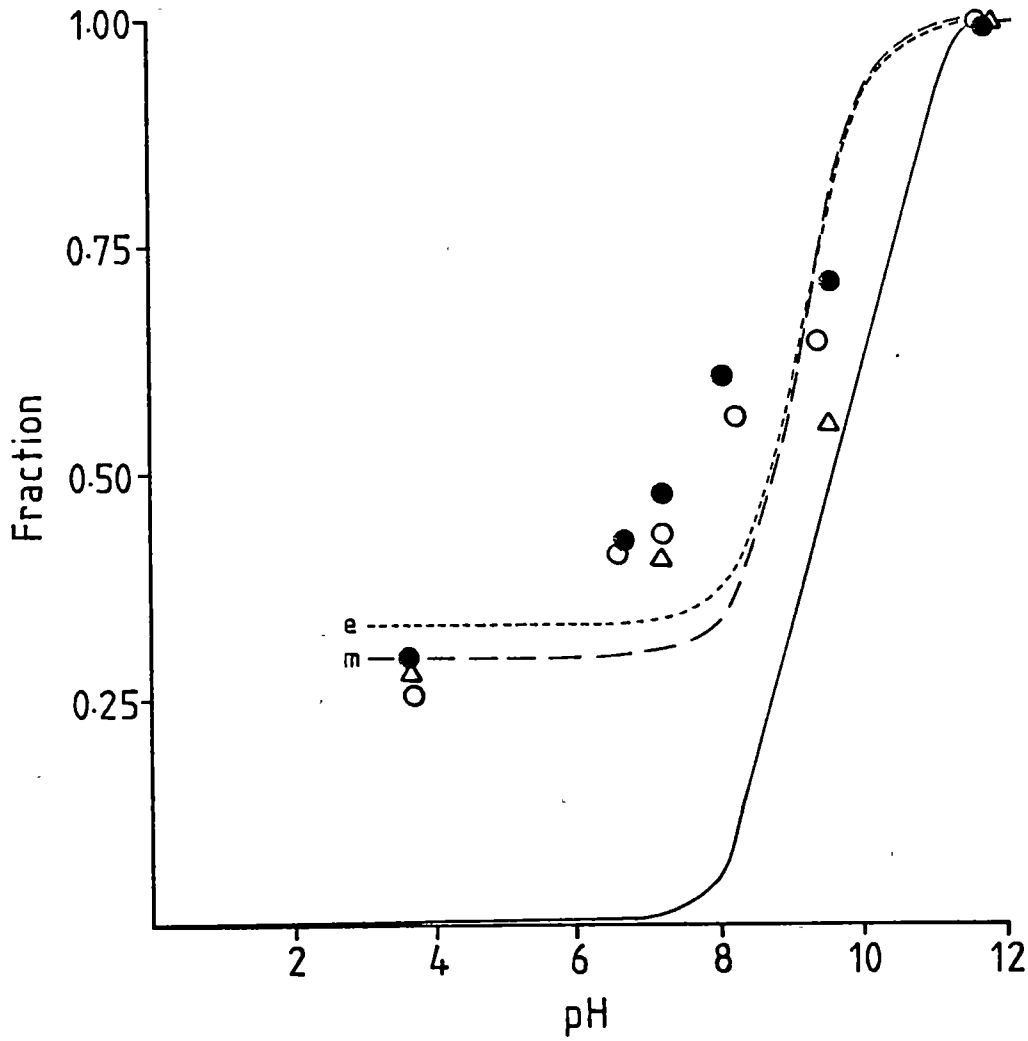


Figure 5.19 Effect of vehicle pH on the steady state flux (J_{ss}) and partition coefficient for ephedrine hydrochloride. The solid line was predicted by DM I. The broken lines were predicted by DM III for the stratum corneum (e) and the inert membrane (m). ○ J_e-LR; △ J_m-LR and ● K.

the inert membrane was generally higher than that obtained for the human stratum corneum (Tables 5.7 and 5.8). The lag times for the inert membrane increased with increase in ionisation but the magnitude of the changes was much smaller than those obtained for the permeation of weak electrolytes through the human stratum corneum.

Partition coefficient

Figures 5.14 to 5.19 show that the apparent octanol-buffer partition coefficient (K') for the total concentration (unionised + ionised) of weak electrolytes was dependent upon pH and the type of buffer system used (Table 5.5); K' values decreased with increase in ionisation of the substance in aqueous solutions.

Table 5.9 shows that the apparent octanol-water partition coefficient (K'') for the weak electrolyte - counter ion complex (rubidium or chloride) were generally found to increase with increase in ionisation, while K'' for rubidium or chloride alone was found to be independent of ionisation.

5.3 Discussion

5.3.1 Methotrexate

Ionic transport

The present work has shown that the permeation of methotrexate through the human stratum corneum from aqueous solutions, over a range of pH's, can only be adequately modelled if the permeation of ionised species is assumed to occur. In previous diffusion (Table 2.5) and compartmental (Table 2.6) models ionic transport

Table 5.7 Steady state flux ($\mu g\ cm^{-2}\ hr^{-1}$) for the permeation of weak electrolytes through human stratum corneum using DM III and linear regression (LR).

	Salicylic acid			Aspirin			Lignocaine hydrochloride			Chlorpromazine hydrochloride			Chlorpheniramine maleate			Ephedrine hydrochloride			Pilocarpine hydrochloride		
pH	f_u	DM III	LR	f_u	DM III	LR	f_u	DM III	LR	f_u	DM III	LR	f_u	DM III	LR	f_u	DM III	LR	f_u	DM III	LR
3.4[e]	-	-	-	0.56	5.14	4.50	0.0001	35.00	33.00	-	-	-	-	-	-	-	-	-	-	-	-
3.6[e]	0.20	8.96	10.00	-	-	-	-	-	-	a	0.34	0.14	a	1.98	2.00	a	24.60	20.00	0.0003	0.26	0.32
5.0[e]	0.01	2.78	9.07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5.2[e]	-	-	-	0.02	2.26	3.00	0.002	35.04	39.00	a	0.34	0.36	a	1.98	2.10	-	-	-	-	-	-
5.8[e]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6.6[e]	a	2.41	3.80	a	2.16	2.15	-	-	-	-	-	-	-	-	-	a	28.65	30.00	-	-	-
7.2[e]	a	2.4	0.48	a	2.15	2.0	0.166	38.16	44.00	0.01	0.34	0.38	0.1	2.02	2.20	0.004	28.90	32.00	-	-	-
8.0[e]	a	2.4	0.30	a	2.15	2.0	0.557	45.59	45.00	-	-	-	-	-	-	0.024	30.80	43.50	0.9990	4.70	5.00
8.4[f]	-	-	-	-	-	-	-	-	-	0.11	0.35	0.40	0.15	2.58	2.80	-	-	-	-	-	-
9.4[f]	-	-	-	-	-	-	0.969	53.42	48.20	0.56	0.40	0.47	0.64	4.51	4.50	-	-	-	-	-	-
9.6[f]	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.500	49.08	48.00	-	-	-
11.7[f]	-	-	-	-	-	-	0.999	54.00	52.50	0.99	0.54	0.64	0.99	5.93	6.00	0.992	73.18	74.00	-	-	-

Flux for DM III calculated using values shown in Table 5.4.

f_u is the fraction unionised.

a - f_u is < 0.0001.

e and f are the type of buffers shown in Table 3.2.

Table 5.8 Steady state flux (μg cm^{-2} hr^{-1}) for the permeation of weak electrolytes through an inert membrane using DM III and linear regression (LR).

pH*	Methotrexate		Salicylic acid		Aspirin		Lignocaine hydrochloride		Chlorpromazine hydrochloride		Chlorpheniramine maleate		Ephedrine hydrochloride	
	DM III	LR	DM III	LR	DM III	LR	DM III	LR	DM III	LR	DM III	LR	DM III	LR
3.4[e]	0.76	0.82	-	-	5.6	5.8	38.00	34.00	-	-	-	-	-	-
3.6[e]	0.73	0.78	9.82	11.20	-	-	-	-	0.30	0.14	3.23	2.45	25.20	23.70
5.0[e]	0.25	0.28	4.00	7.50	-	-	-	-	-	-	-	-	-	-
5.2[e]	-	-	-	-	3.24	4.0	38.06	44.00	0.30	0.50	-	-	-	-
5.8[e]	0.17	0.18	-	-	-	-	-	-	-	-	-	-	-	-
6.6[e]	-	-	3.69	3.70	3.20	3.8	-	-	-	-	-	-	-	-
7.2[e]	-	-	-	-	-	-	-	-	-	-	3.33	3.32	25.44	34.10
8.0[e]	0.16	0.13	3.69	0.63	3.15	2.61	53.61	45.00	-	-	-	-	-	-
9.4[f]	-	-	-	-	-	-	-	-	0.57	0.57	3.40	7.50	-	-
9.6[f]	-	-	-	-	-	-	-	-	-	-	-	-	54.90	47.00
11.7[f]	-	-	-	-	-	-	66.00	71.30	0.78	0.76	8.40	8.00	84.14	82.20

e and f are the symbols for the buffer systems shown in Table 3.2

*pH measured at 25°C.

Table 5.9 Effect of vehicle pH on the apparent partition coefficient (K'') of weak electrolytes using radioactive counter ion (mean of two measurements).

pH*	using rubidium ($K'' \times 10^1$)							using chloride ($K'' \times 10^1$)								
	Without drug	Methotrexate		Salicylic acid		Aspirin		Without drug	Lignocaine hydrochloride		Chlorpromazine hydrochloride		Chlorpheniramine maleate		Ephedrine hydrochloride	
		f_i	K''	f_i	K''	f_i	K''		f_i	K''	f_i	K''	f_i	K''	f_i	K''
2.0[e]	-	0.005	3.9	-	-	-	-	-	-	-	-	-	-	-	-	-
3.0[e]	-	0.048	3.8	-	-	-	-	-	-	-	-	-	-	-	-	-
3.4[e]	-	0.113	4.0	-	-	0.443	4.0	2.0	0.999	6.6	-	-	-	-	-	-
3.6[e]	3.6	0.168	4.2	0.799	3.1	-	-	-	-	-	-	-	-	-	-	-
5.0[e]	-	0.868	4.4	-	-	-	-	-	-	-	-	-	0.999	4.0	-	-
5.2[e]	4.1	-	-	0.924	3.5	0.980	4.2	-	0.998	4.6	0.999	4.2	-	-	-	-
5.8[e]	-	0.989	5.0	-	-	-	-	-	-	-	-	-	-	-	-	-
6.6[e]	-	-	-	0.999	6.0	0.999	4.4	-	-	-	-	-	-	-	0.999	2.4
7.2[e]	-	0.999	4.8	>0.99	5.0	>0.99	4.5	1.8	0.834	4.8	0.992	4.0	0.989	4.1	0.996	2.1
8.0[e]	3.9	0.999	4.7	>0.99	5.0	>0.99	4.4	-	0.443	2.1	-	-	0.941	2.2	0.976	2.0
8.4[f]	-	-	-	-	-	-	-	-	-	-	0.888	5.0	-	-	-	-
9.4[f]	-	-	-	-	-	-	-	-	-	2.0	0.443	2.4	-	-	-	-
9.6[f]	-	-	-	-	-	-	-	-	-	-	-	-	0.340	2.3	0.500	2.0
11.7[f]	-	-	-	-	-	-	-	2.2	-	2.0	0.004	2.1	0.001	2.0	0.008	2.0

e and f are the symbols for the buffer systems shown in Table 3.2.

f_i is the fraction of the ionised weak electrolytes present in the aqueous solutions.

*pH measured at 25°C.

has been neglected. Both diffusion model III (Figure 5.4) and compartmental model II (Figure 5.3) gave good regression fits, indicating that the ionised species of methotrexate can permeate the human stratum corneum. Further evidence for ionic transport of methotrexate is shown in Figure 5.5. The deviation, in the steady state flux of methotrexate (for both human skin and the inert membrane), from the prediction of the pH-partition hypothesis, also referred to as the pH shift (Wagner 1975), is consistent with the transport of ionised methotrexate across these membranes.

pH shift

The pH shift is based upon the barriers to penetration either before or after the biological membranes (Wagner 1975). Roberts et al (1978) suggested that an aqueous boundary diffusion layer existed adjacent to the excised skin and this affected the permeation of lipophilic phenols. These authors used the change in permeability coefficient with temperature to demonstrate the boundary layer phenomenon. A pH-shift is consistent with aqueous boundary layers and partially contributes to the overall diffusional resistance of the skin (Figure 5.5). In the present study the quantitative extent of the boundary layer contribution to the overall diffusion resistance was not ascertained.

Evidence for ion pair transport

Ionised species do not have favourable free energies for transport across predominantly lipid stratum corneum and it was suggested previously that it is only the unionised form of the

drugs which can penetrate the skin in significant amounts (section 2.3.1.7). It is possible, however, that ionised compounds may be absorbed from the gastrointestinal tract by an ion pair mechanism (Higuchi 1971). More recently there has been some evidence of the possibility of ion pair transport through human skin (Barker and Hadgraft 1981).

Since methotrexate is predominantly anionic, it is possible that it will form an ion pair with cations present in the vehicle. The relevant partition coefficients, K' and K'' (Figures 5.5 and 5.6), reflect the possible formation of an ion pair between anionic methotrexate and the counter ion present in the vehicle. The partition coefficient (K') is however found to decrease with increase in ionisation (Figure 5.5). As these values are based upon total drug concentration, it is difficult to identify the formation of ion pairs but there is a clear indication of the effect of buffer constituents on the values of K'. The use of organic counter ions did not however have a marked effect on the values of K' (Table 5.4).

The steady state flux of methotrexate was found to change by approximately two fold, when different buffers were used at the same pH values. The partition coefficients (K'') and permeability coefficients of the rubidium-methotrexate complex (pH 3.4 and 8.1), measured with rubidium, were found to increase with increase in the extent of ionisation of methotrexate (Figure 5.6), which can be considered as evidence for the formation of an ion pair between the anions of methotrexate and the counterion rubidium.

Assuming that the above phenomenon occurs in the presence of other cations, in the vehicle containing methotrexate, then it follows that methotrexate will form an ion-pair with cations present in the buffer and that lag times will be anticipated to be higher for this polar ion-pair. This ion-pair will however be more lipophilic than the ionised species of methotrexate because the charges on the ionic methotrexate would be neutralised due to the anionic methotrexate and counter ions present in the buffer systems. It is more likely therefore that this ion-pair will cross the lipid stratum corneum ("intracellular" route) with relative ease as the predominantly lipophilic nature of human stratum corneum could act as a proton donor. The long lag times predicted with increase in ionisation (Table 5.3) are also consistent with the possible transport of ionic methotrexate through human stratum corneum as ion-pairs.

This phenomenon is more likely to occur when methotrexate is in a highly ionised form. The ease with which the ion pair would partition into the lipid phase of the stratum corneum would vary with the type of cations present in the buffer system. The variation is seen in the values of partition coefficients (K') measured during this work (Table 5.4). The rate of penetration through the inert membrane also supports this hypothesis. Although the flux for the inert membrane was dependent upon ionisation (Table 5.7), there was only a very small increase in the lag time with change in pH. This indicates that the lipophilic nature of the inert membrane facilitates the transport of the ionic species possibly as ion pairs.

The type of buffer constituents present in the vehicle also had an influence on the steady state flux of methotrexate (Tables 5.3, 7.2 and Figure 5.2). The greater number of sodium ions in buffer-c (Table 3.2) gave a higher value of flux at pH 5.8 and 8.1 in comparison with the flux obtained when buffer-d or buffer-e (Table 3.2) was used as a vehicle. This shows that the presence of a high number of sodium ions might allow for the formation of an ion pair with relative ease, which in turn will increase the overall flux of ionic species of methotrexate. High flux obtained for buffer-a in comparison to buffer-e at pH 5.0 for methotrexate could be due to a similar reason as discussed above.

Dependence of lag time on pH and mechanism of ionic transport

The raw data for the in vitro permeation of methotrexate indicates that the lag time increases with increase in ionisation (Table 5.3). Blank and Scheuplein (1969) have suggested that the magnitude of the lag time may reflect the route of penetration of solutes through the skin. Long lag times may reflect the transport of solute through the shunts, prior to the establishment of the steady state of skin penetration. This hypothesis is mainly based on the permeation of steroids through the human skin (Chapter 4).

Although it has been known that individual ions or electrolytes can penetrate the skin (Tregear 1966a; Schaefer et al 1982), there is no physical evidence regarding the significance of "shunts" during the transport of ions and/or ionised species

through human skin, as Wahlberg (1968a) and Middleton (1969) have suggested that electrolytes are more likely to penetrate via the "intracellular" route. In the case of methotrexate the reduction in lag time with decrease in ionisation indicates that it might be the unionised drug which passes through the "intracellular" route, while the ionised species might permeate via the "shunts" with negligible lag time. However, the present work indicates that the ionised species may not permeate through the shunts; indeed it is more likely that the ionic species of methotrexate permeates through the "intracellular" route of the skin in the form of ion pairs, as discussed above.

Choice of models

Diffusion model II was found to predict an extremely long lag time (> 100 hours) for methotrexate with the permeability coefficient for "shunts" (kp_s) approaching a value of 0.00033 (percentage response explained = 40.67). Diffusion model II was also unable to adequately describe the methotrexate data at pH values where the substance was in a highly ionised form. Therefore it was considered that this model is inappropriate to describe the transport of methotrexate through excised human stratum corneum.

In case of diffusion model I and compartment model I non-linear regression fits for the experimental data are only found for pH 3.4 and 3.6 when methotrexate is about 10% ionised. At higher pH's, with an increase in the extent of ionisation, the experimental data was poorly described by the model (Figures 5.3 and 5.4). The discrepancy between this work and that of Wallace

and Barnett (1978), could be due to the nature of the experimental conditions as Wallace et al (1978) had used hairless mouse skin. Their conclusions, based upon compartmental analysis, that the penetration of methotrexate through the "shunt" pathway increased as the vehicle pH and the extent of ionisation increased (Table 2.6), cannot be supported by the results obtained in the present in vitro studies using either the diffusion or the compartmental models, even though the fractional area of the "shunts" would be greater in human skin than in hairless mouse skin. The fast rate of penetration through the mouse skin (section 2.3.2) could be the reason for the discrepancy between the experimental data used by Wallace and Barnett (1978) and that obtained in the present work, as it is possible that the high permeability of animal skin could imply the presence of a "shunt" pathway. The data obtained for the pH dependence of steady state flux (Table 5.3) was however consistent with the results reported for full thickness mouse skin (Wallace et al 1978).

The mathematical models (DM III and CM II) were unable to correctly predict the lag times of methotrexate. A high %CV was obtained for the final parameter estimates of the lag times (Tables 5.1 and 5.2). However, the unionised and the ionised permeability coefficient (Table 5.1) and k_{12} (Table 5.2) were predicted with a low %CV. Boxenbaum et al (1974) have discussed this problem in the estimation of parameters for mathematical models and suggested that such a situation does not necessarily result from poor data, as it is possible to obtain good computer fits but with large standard deviations. This indicates that it

may be more appropriate to describe the dependence of lag time of methotrexate permeation through excised human stratum corneum, on the degree and extent of ionisation in solution using linear regression approach (Figure 2.3).

5.3.2 Compounds other than methotrexate

Ionic transport

The permeation of weak electrolytes, other than methotrexate, through human stratum corneum from aqueous solutions over a range of pH's can only be adequately modelled when ionic transport is assumed to occur, as was found for methotrexate. The diffusion model III gave considerably better fits than diffusion model I for the majority of weak electrolytes (Figures 5.7 to 5.13; Table 5.5). The phenomenon of pH-shift (Wagner 1975) was also observed for other weak electrolytes (Figures 5.14 to 5.19). These results could be considered as evidence for the transport of ionic species of weak electrolytes through excised human stratum corneum.

Evidence and mechanism of ion pair transport

The partition coefficients (K' and K'') also reflect the possible formation of ion pairs between the weak electrolytes and counter ions present in the vehicle (Figures 5.13 to 5.19; Table 5.9). The partition coefficients (K') were found to decrease with increase in ionisation (Figures 5.13 to 5.19). All figures show a close relationship between the fraction change in the flux and pH for both the stratum corneum and the inert membrane. These fractional changes correlated well with fractional change in

partition coefficient for majority of weak electrolytes. However, for some substances the values of the partition coefficient deviate from the line predicted by DM III. This discrepancy could be due to the difficulty in accurately measuring the partition coefficients of any substance especially weak electrolytes where the small change in pH value may alter the magnitude of partition coefficients. The use of organic counter ions had a small effect on the values of K' (Table 5.4). It appears that the partition coefficient (K') is dependent upon the pH of the aqueous solution, that is degree of ionisation, rather than the type of organic counter ions present in the vehicle (Table 5.4). Similarly the inorganic counter ions present in the buffer system also had a small effect on the partition coefficients of the weak electrolytes (Table 5.4). In the case of weak electrolytes with basic pK_a values and at pH 8.1, it was buffer d and e (Table 3.2) which gave a relatively higher K' values in comparison to buffer-c (Table 5.4). This could be due to the balance of charges i.e. more neutral species of weak electrolytes are present, when buffer system d or e (Table 3.2) was used as a vehicle. However, there is no definitive evidence to substantiate this hypothesis.

The partition coefficient measured with an appropriate radioactive counter ions (K'') generally showed an increase with increase in ionisation (Table 5.9). In the cases of chlorpromazine, chlorpheniramine and ephedrine, the partition coefficient (K'') only increased when the weak electrolyte was around 75% ionised in aqueous solutions (Table 5.9). The permeability coefficients based upon radioactive counter ions

(rubidium or chloride) were generally found to increase with increase in ionisation while the permeability coefficient of the radioactive counter ion was found to be independent of ionisation. These results could also be considered as evidence of formation of ion pairs between ionic weak electrolytes and counter ions present in the vehicle.

It is more likely that the bulk transport of highly ionised weak electrolytes may also occur through the "intracellular" route of excised human stratum corneum with a mechanism similar to that described for methotrexate. The general increase in the lag times with the increase in ionisation (Figures 5.7 to 5.13) also supports this hypothesis. Most of the substances used here (except methotrexate) do not have very long lag times and it is possible therefore that the ion pairs of weak electrolytes may also pass through the "shunts" when a substance has a short lag time, that is when the compounds are approximately 50 to 60% ionised in aqueous solutions. But when the weak electrolyte is more than 90% ionised the bulk transport is more likely to be by the "intracellular" route. This hypothesis could be further substantiated by the presence of small fractional area of "shunts" (section 2.2.1), scarcity of hair follicles and effect of hydration (as discussed in Chapter 4), especially in the excised abdominal human skin used during the present in vitro studies.

The type of buffer constituent present in the vehicle had a very small effect on the steady state flux of the basic weak electrolytes. However, the permeation of salicylic acid and

aspirin increased by approximately 20%, when buffer d or e (Table 3.2) was used as a vehicle. This also shows that an appropriate counter ion may enable ionic species of weak electrolytes to form an ion pair with relative ease, which in turn may influence the overall flux of ionic species of the substance.

Reliability of diffusion model III

Diffusion model III gave good regression fits for the permeation of weak electrolytes through human stratum corneum in comparison with the other diffusion models used in this work (Table 5.5). It also adequately described the data obtained for the permeation of weak electrolytes through an inert membrane (Table 5.6). Boxenbaum et al (1974) have discussed the problems usually encountered during the estimation of final parameters for mathematical models. Their results show that such problems do not necessarily result from poor data, as it is possible to obtain good computer fits with large standard deviations in parameter values. The inability of the model to predict lag times for some of the weak electrolytes may be a reflection of this problem. Generally, large standard deviations were obtained for some of the final parameters used to estimate the steady state fluxes and lag times. This indicates that it is difficult to estimate the correct trend in the the lag times by diffusion model III for weak electrolytes which penetrate the human stratum corneum and inert membrane at a fast rate. The use of weighting (y^{-1} or y^{-2}) did not improve the fit nor did it predict the parameter values which allowed the correct estimation of lag times and the steady state fluxes for the permeation of weak

electrolytes through the human stratum corneum.

On the other hand the short lag times predicted by diffusion model III may also indicate that the ionised species of these compounds permeate through excised stratum corneum at a very fast rate. The lag times obtained for the weak electrolytes (using the radioactive counterion - weak electrolyte complex method) were found to be negligible for the majority of the substances. It may be possible that the permeation of the counterion complex, with negligible lag times, may be an indication of penetration of ion pairs through "shunts". It follows that the incorrect results predicted by diffusion model III for weak electrolytes with short lag times could be due to the fast penetration of these ion pairs, which the diffusion model III is unable to correctly predict.

The F-test (Appendix 3) cannot be considered as a reliable means of indicating the superiority of one model over another. It is also important to compare the regression fits, correlation coefficients and residual plots when comparing various mathematical models rather than relying only on an F-test (Boxenbaum et al 1974; Pedersen 1977).

Conclusions

The results of this section of the work support the hypothesis that the ionised species of methotrexate and other weak electrolytes can permeate the human stratum corneum. Michaels et al (1975) used a mathematical model to predict that the unionised species of ephedrine, chlorphenaramine and scopolamine generally

achieved a greater degree of permeation than did the ionised species. There was no substantial evidence obtained in the present work to support the hypothesis put forward by Michaels et al (1975). However, the recent work of Swarbrick et al (1984) has shown that both ionised and unionised species of carboxylic acids can permeate excised human skin.

The formation of an ion pair and its ability to partition into the lipid phase with relative ease (Higuchi 1971) indicates that weak electrolytes are likely to penetrate the excised human stratum corneum via the "intracellular" route, when the substance(s) is in a highly ionised form. However, it may be possible that the transport of unionised species occurs via "shunts" and through the "intracellular" route in vivo (Barry 1983). The present results also show that the permeation of weak electrolytes can be adequately described by a mathematical model which incorporates factors for the transport of both the ionised and unionised species present in a vehicle containing the drug.

CHAPTER 6

Iontophoresis and percutaneous absorption

Ions have been shown to penetrate the skin when an electrical potential gradient is applied and this process is referred to as iontophoresis (Harris 1967). Iontophoresis is a simple, safe and well documented method of introducing ions or polar substances into the skin by use of a direct current of 40 μA to 1 mA applied through electrodes placed on the surface of the skin. (Gangarosa et al 1978, 1980; Schaefer et al 1982; Wahlberg 1970; Harris 1967).

Russo et al (1980) found that when lignocaine was administered topically to human volunteers using iontophoresis it produced a local anaesthesia of significantly longer duration than did administration by swabbing, thus avoiding the use of hypodermic needles. The optimal conditions for iontophoretic transport of lignocaine were not determined by Russo et al (1980).

Pilocarpine is also administered topically by iontophoresis to induce sweating so that chloride concentration in the sweat can be measured to enable the screening of especially new born babies for cystic fibrosis (Gibson and Cooke 1959; Kopito and Shwachman 1969). The pilocarpine (solution pH 5.0) iontophoresis method only requires five minutes, to induce sweating, in comparison to a plastic suit method which takes thirty to ninety minutes (Kopito and Shwachman 1969). The application of external heat by electrodes, has also been used for stimulation of sweat (Warwick and Hansen 1965). The plastic suit method causes the patient discomfort and heat stress, while with the heat stimulation method there is a possibility of burning the patient (Clark et al

1961; Warwick and Hansen 1965).

In the present section, the role of pH and the effect of iontophoresis on the rate of *in vitro* permeation of weak electrolytes through human stratum corneum is reported.

6.1 Experimental

The permeation studies, with and without iontophoresis, were carried out using the permeation cells and the technique described in sections 3.3.1, 3.4 and 3.6. The methods of analysis for the nine weak electrolytes are shown in Table 3.1. The steady state flux, lag times and the permeability coefficients were calculated using the method described in section 2.5 (Figure 2.3 and equation 2.5). The iontophoresis data was fitted to diffusion model III (Chapter 5).

The fraction change in flux was defined as the ratio: (flux during iontophoresis - normal flux)/flux during iontophoresis. The linear regression values of steady state flux were used to calculate this fraction change.

6.2 Results

Figures 6.1 to 6.8 shows the cumulative amount of weak electrolyte permeated through excised human stratum corneum under various pH conditions and in the presence of iontophoresis. The solid lines were predicted by diffusion model III, using the experimental data points shown in the Figures. In the absence of iontophoresis the amount of the substance passing through the stratum corneum in a given time decreases as the degree of

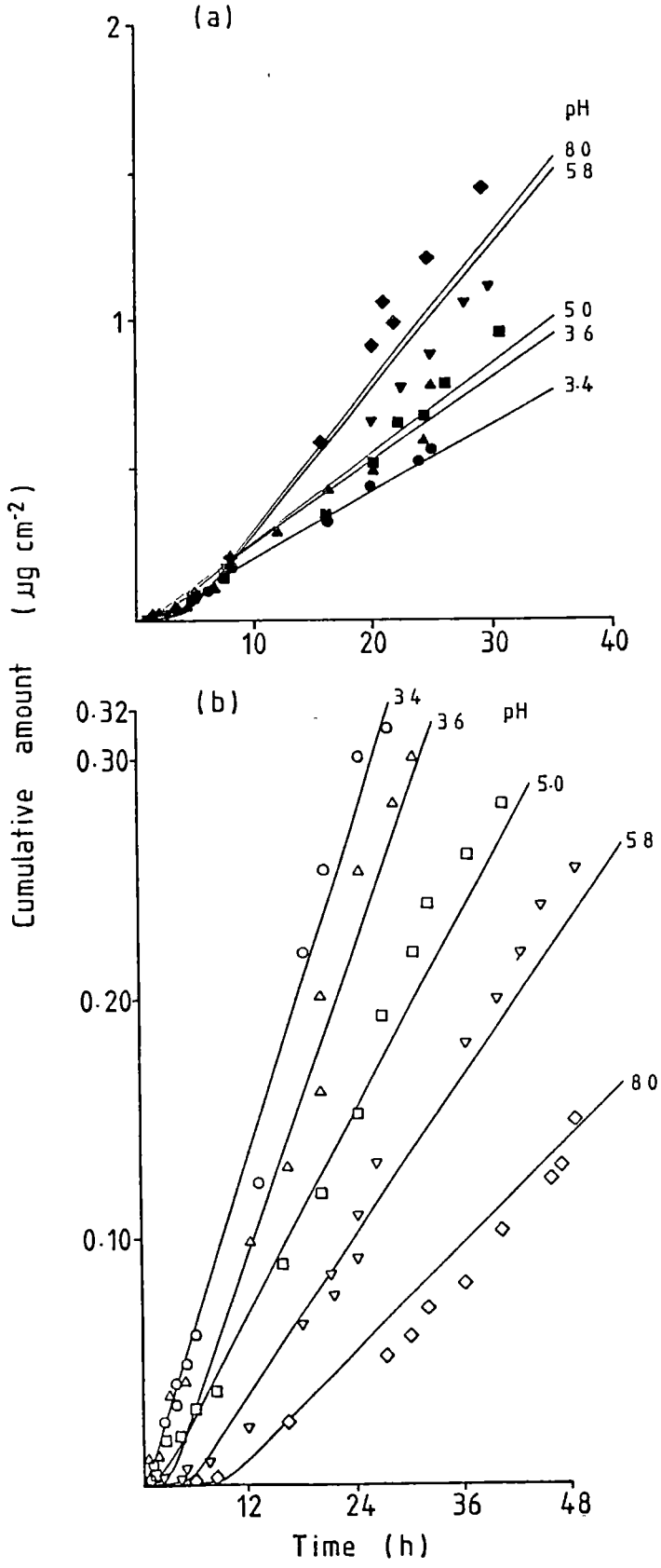


Figure 6.1 Permeation of methotrexate through the human stratum corneum. The solid line is the result predicted by DM III. (a) with iontophoresis, (b) without iontophoresis. ● ○ pH 3.4, ▲ △ pH 3.6, ■ □ pH 5.0, ▼ ▽ pH 5.8, ◆ ◇ pH 8.0.

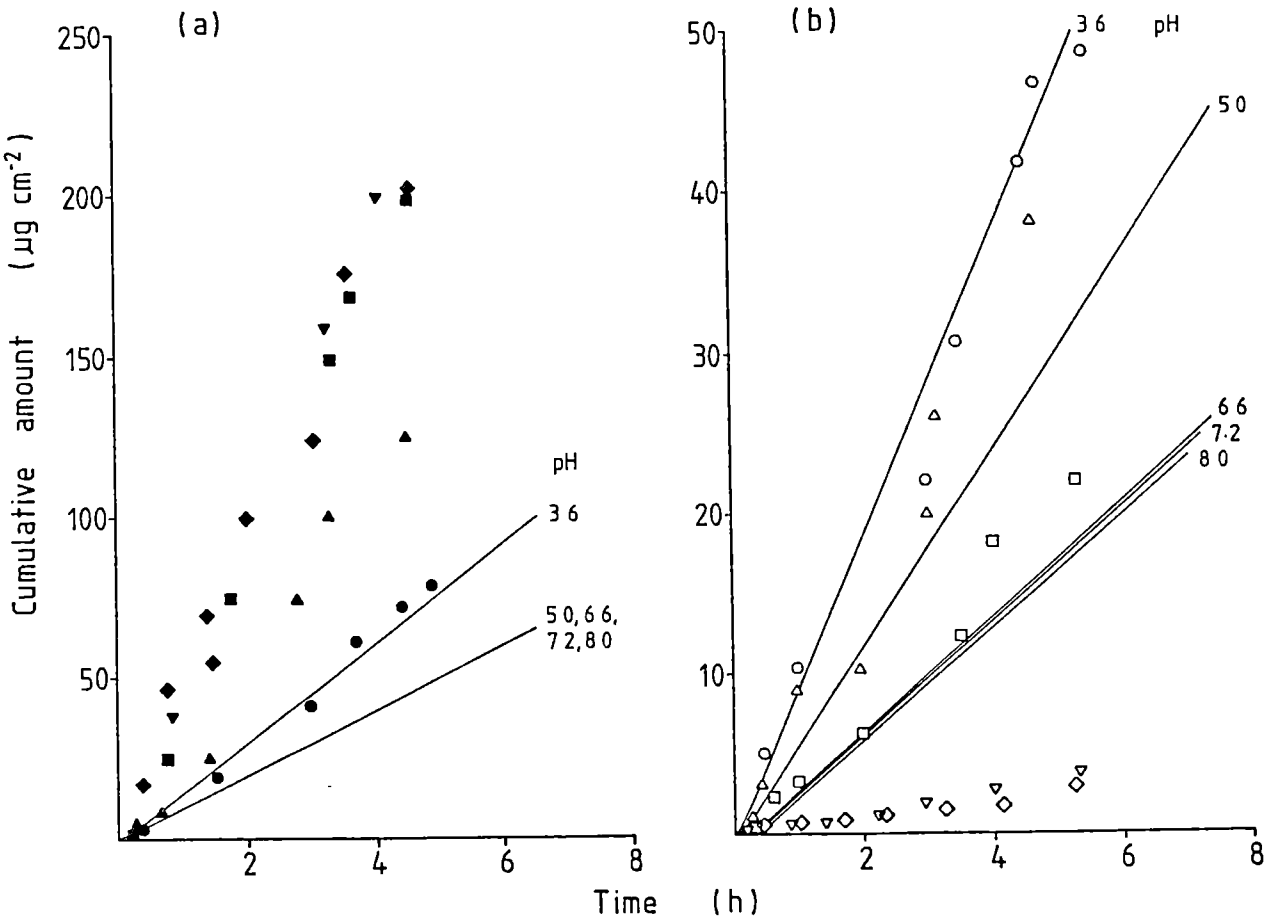


Figure 6.2 Permeation of salicylic acid through the human stratum corneum. The solid line is the result predicted by DM III. (a) with iontophoresis, (b) without iontophoresis. ● ○ pH 3.6, ▲ △ pH 5.0, ■ □ pH 6.6, ▼ ▽ pH 7.2, ◆ ◇ pH 8.0.

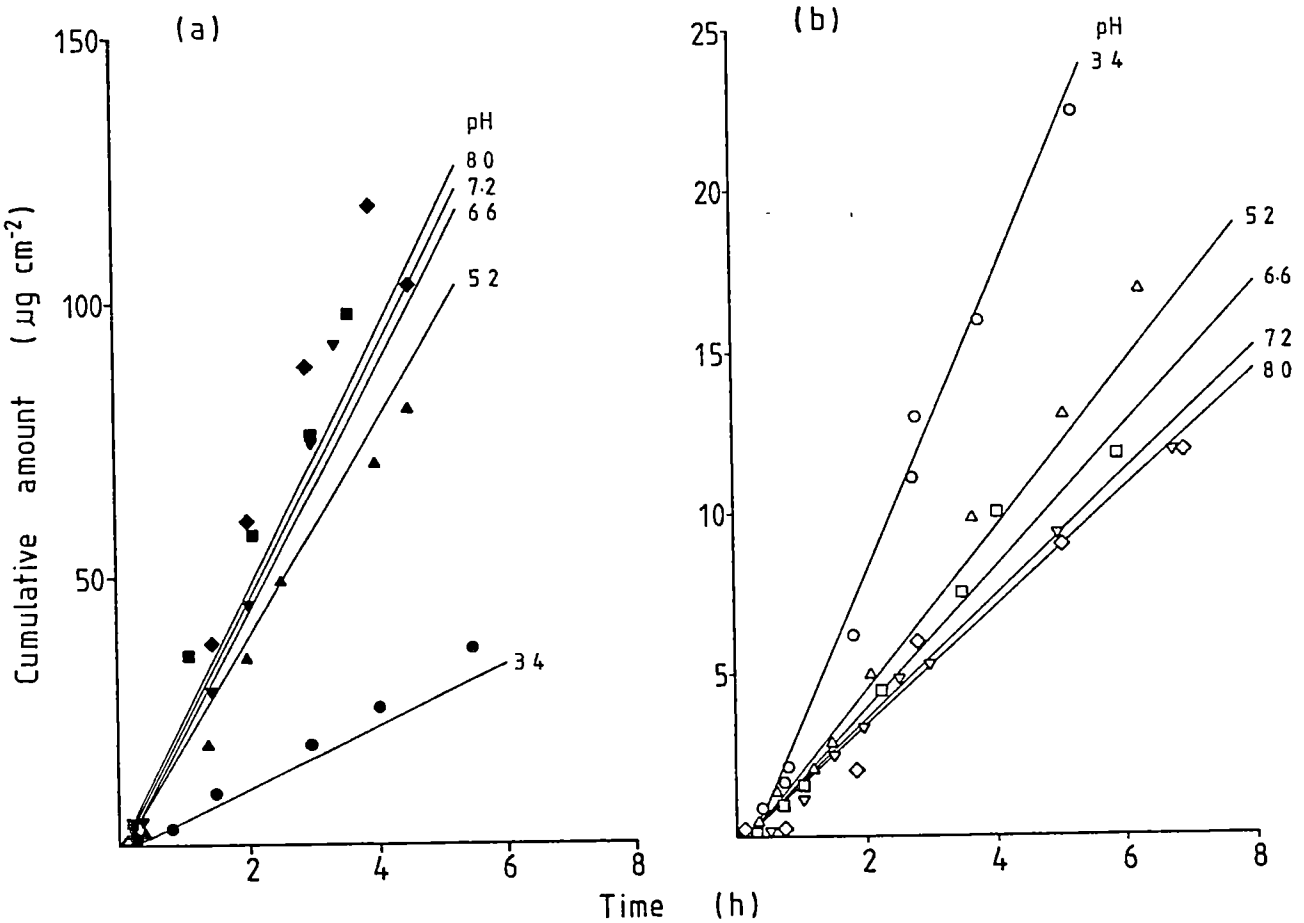


Figure 6.3 Permeation of aspirin through the human stratum corneum. The solid line is the result predicted by DM III. (a) with iontophoresis, (b) without iontophoresis. ● ○ pH 3.4, ▲ △ pH 5.2, ■ □ pH 6.6, ▼ ▽ pH 7.2, ◆ ◇ pH 8.0.

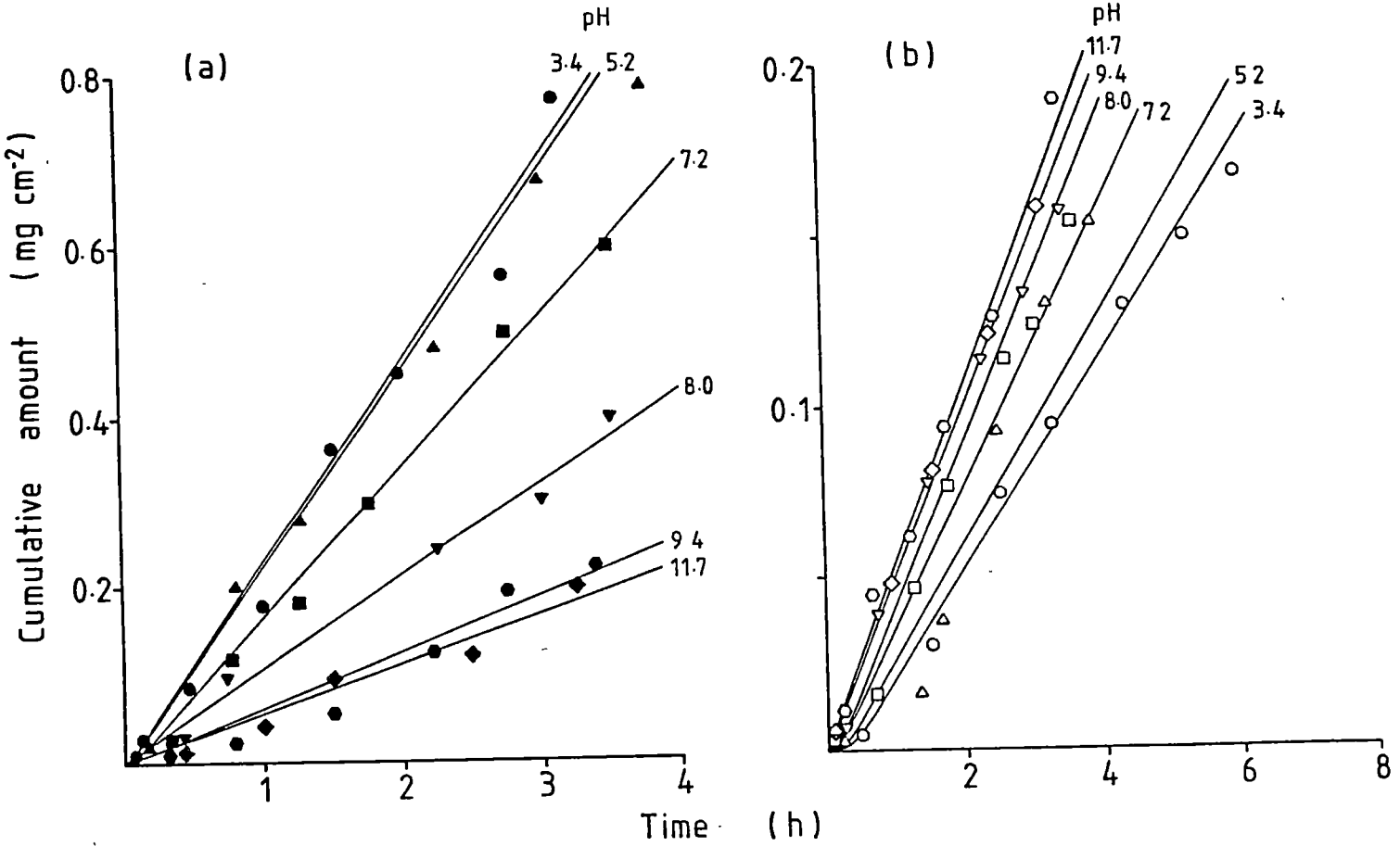


Figure 6.4 Permeation of lignocaine hydrochloride through the human stratum corneum. The solid line is the result predicted by DM III. (a) with iontophoresis, (b) without iontophoresis. ● ○ pH 3.4, ▲ △ pH 5.2, ■ □ pH 7.2, ▼ ▽ pH 8.0, ◆ ◇ pH 9.4, ⬟ ⬡ pH 11.7.

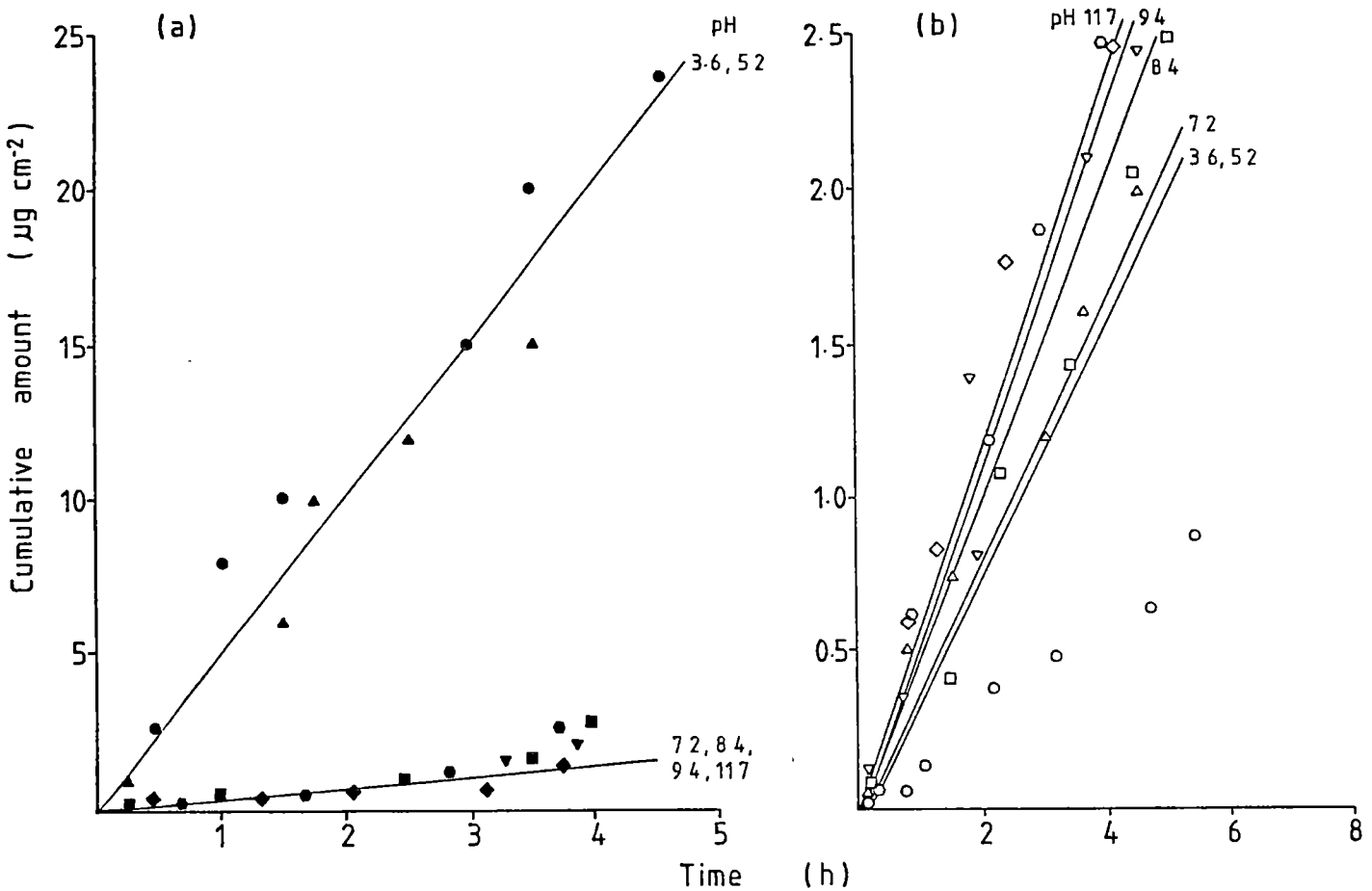


Figure 6.5 Permeation of chlorpromazine hydrochloride through the human stratum corneum. The solid line is the result predicted by DM III. (a) with iontophoresis, (b) without iontophoresis. ● ○ pH 3.6, ▲ △ pH 5.2, ■ □ pH 7.2, ▼ ▽ pH 8.4, ◆ ◇ pH 9.4, ⬢ ⬡ pH 11.7.

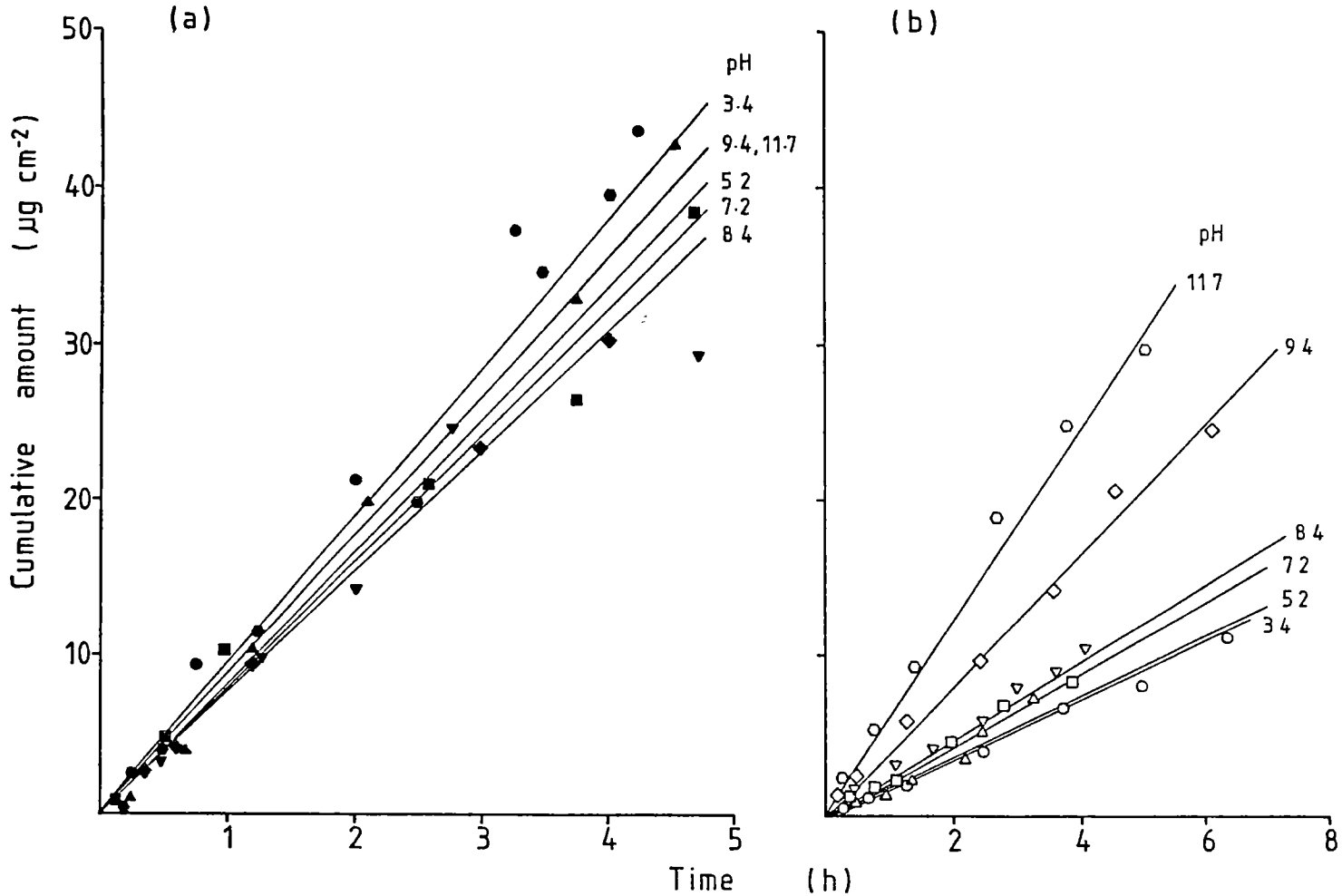


Figure 6.6 Permeation of chlorpheniramine maleate through the human stratum corneum. The solid line is the result predicted by DM III. (a) with iontophoresis, (b) without iontophoresis. ● ○ pH 3.4, ▲ △ pH 5.2, ■ □ pH 7.2, ▼ ▽ pH 8.4, ◆ ◇ pH 9.4, ⬟ ⬠ pH 11.7.

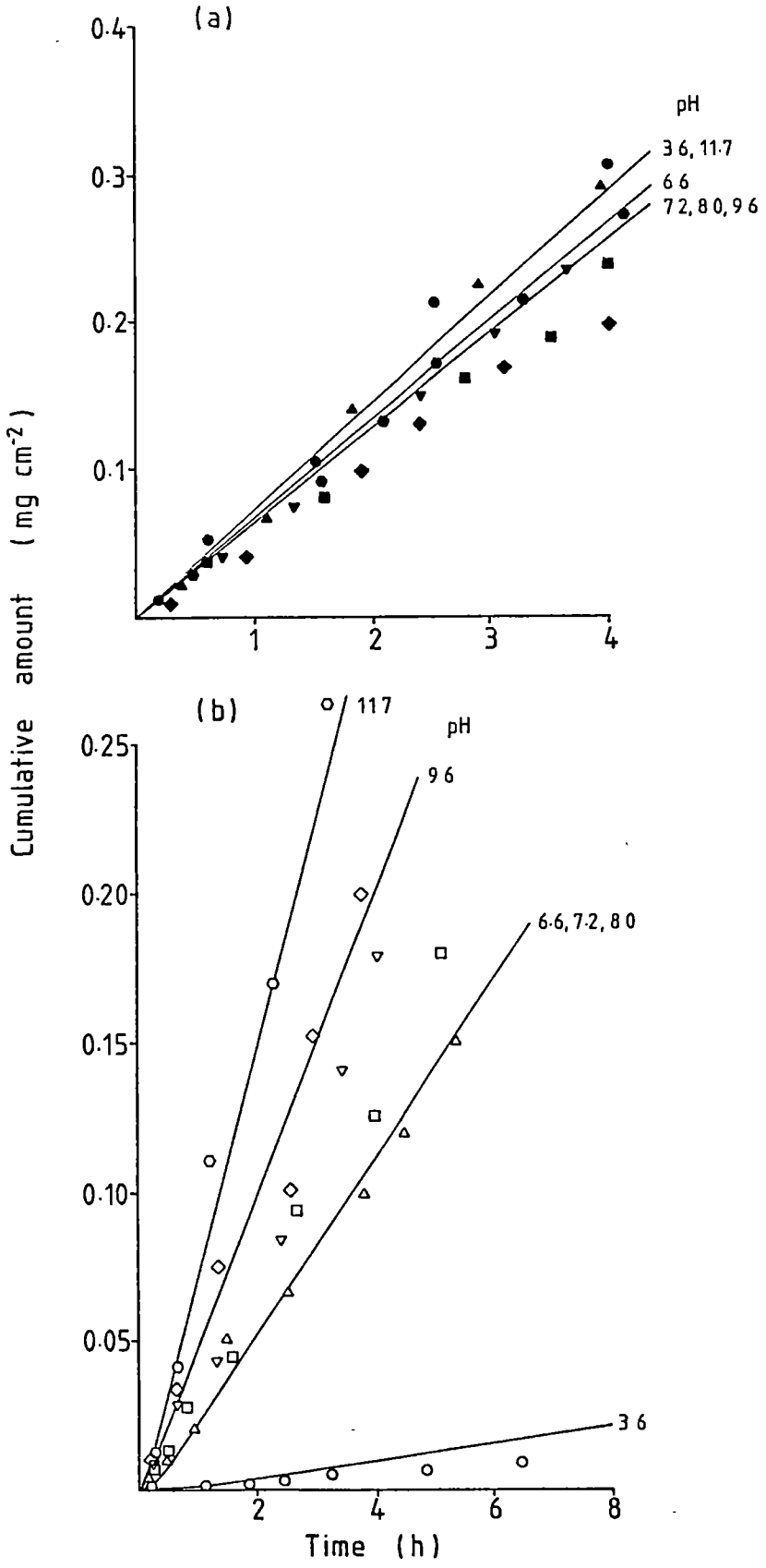


Figure 6.7 Permeation of ephedrine hydrochloride through the human stratum corneum. The solid line is the result predicted by DM III. (a) with iontophoresis, (b) without iontophoresis.
● ○ pH 3.6, ▲ △ pH 6.6, ■ □ pH 7.2, ▼ ▽ pH 8.0, ◆ ◇ pH 9.6, ● ○ pH 11.7.

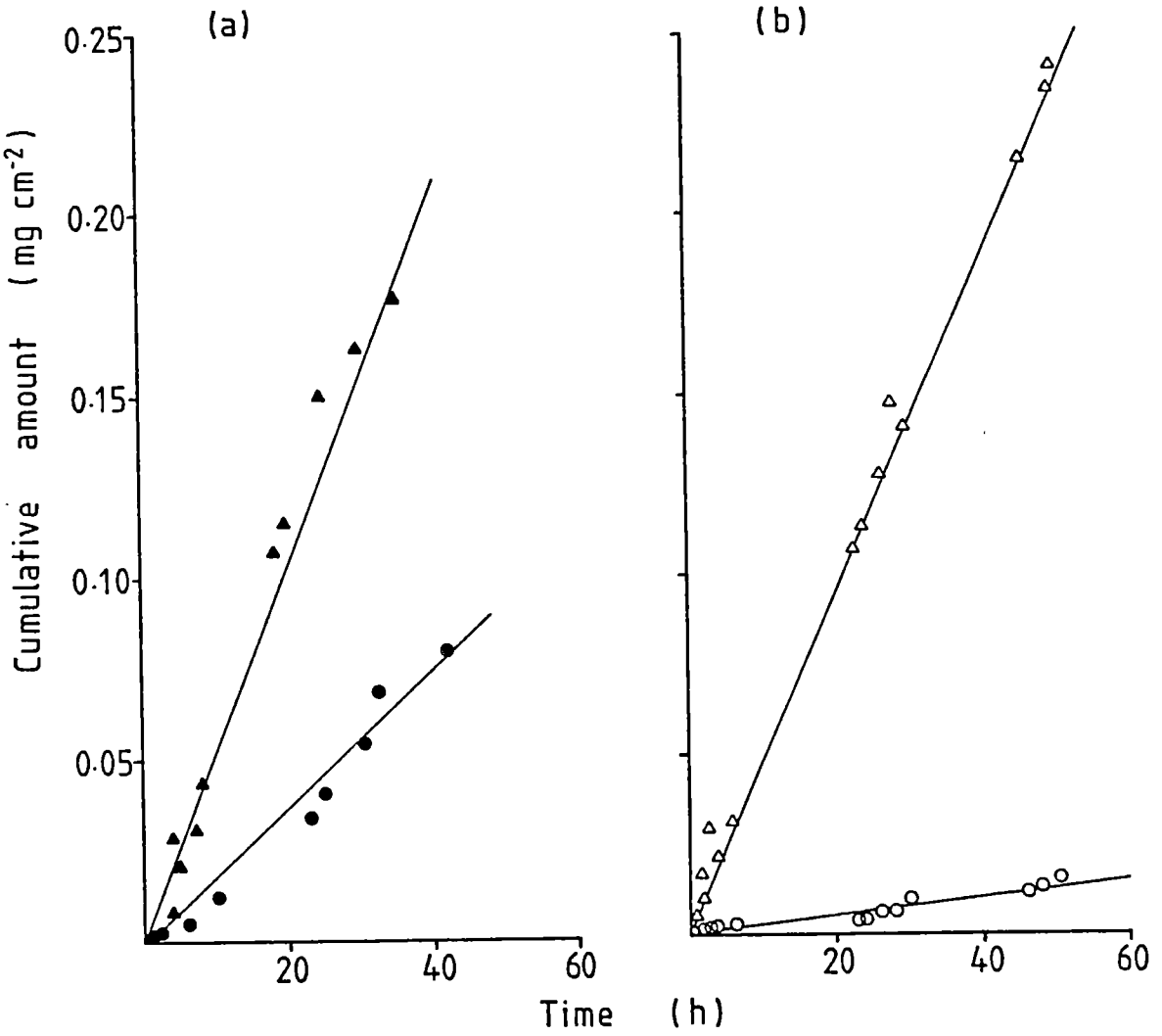


Figure 6.8 Permeation of pilocarpine hydrochloride through the human stratum corneum. The solid line is the result predicted by DM III. (a) with iontophoresis, (b) without iontophoresis. ● ○ pH 3.6, ▲ △ pH 8.0.

ionisation increases with the change in pH of the aqueous solutions. When iontophoresis is applied, the cumulative amount permeated is increased markedly at pH value(s) where the degree of ionisation is high. Figures 6.1 to 6.8 also show that the lag time for the majority of weak electrolytes is very small. Methotrexate (Figure 6.1) and pilocarpine (Figure 6.8) are exceptions.

Table 6.1 shows the unionised and ionised permeability coefficients predicted by diffusion model III. The ionised permeability coefficients were generally found to be higher for the substances when iontophoresis was applied, while the unionised permeability coefficients are of same order as those predicted for the permeation of weak electrolytes without iontophoresis.

Diffusion model III did not correctly predict the unionised and ionised lag times; the reason for this discrepancy has already been discussed in Chapter 5.

Table 6.2 shows that at pH values at which the solute is highly ionised, the steady state flux is found to increase during iontophoresis. This indicates that the flux of weak electrolytes without iontophoresis, is dependent on the degree of ionisation (Chapter 5); the different fluxes with iontophoresis reflect the dependence of the rate of penetration on the extent of ionisation.

Figures 6.9 to 6.15 show that the fraction change in flux for weak electrolytes during iontophoresis is related to the degree

Table 6.1 Parameter values for the permeation of weak electrolytes through the human stratum corneum (with and without iontophoresis) using diffusion model III.

Substance		k_{p_u}	k_{p_i}	t_{L_u}	t_{L_i}	% response explained
Methotrexate	-A	0.0007 (10)	0.0020 (15)	< 0.1	< 0.1	89.22
	-B	0.0004 (10)	0.0001 (12)	0.2400 (368)	4.2700 (80)	80.97
Salicylic acid	-A	0.052 (20)	0.0100 (20)	< 0.1	< 0.1	80.25
	-B	0.0440 (25)	0.0030 (30)	0.1510 (482)	< 0.1	79.59
Aspirin	-A	0.016 (15)	0.056 (7)	< 0.1	< 0.1	89.50
	-B	0.0150 (12)	0.0043 (6)	0.6100 (56)	0.1491 (150)	95.47
Lignocaine hydrochloride	-A	0.0350 (61)	0.214 (6)	< 0.1	< 0.1	82.85
	-B	0.0540 (8)	0.0350 (7)	< 0.1	0.1400 (127)	93.64

Table 6.1 (continued)

Substance		k_{p_u}	k_{p_i}	t_{L_u}	t_{L_i}	% response explained
Chlorpromazine hydrochloride	-A	0.0080 (10)	0.0770 (12)	< 0.1	< 0.1	79.29
	-B	0.0065 (9)	0.0057 (14)	< 0.1	< 0.1	79.17
Chlorpheniramine maleate	-A	0.0098 (12)	0.0160 (6)	0.3000 (35)	< 0.1	92.45
	-B	0.0099 (2)	0.0033 (4)	0.1520 (50)	< 0.1	99.11
Ephedrine hydrochloride	-A	0.1040 (20)	0.1143 (23)	< 0.1	< 0.1	70.20
	-B	0.1226 (14)	0.0410 (9)	< 0.1	< 0.1	79.03
Pilocarpine hydrochloride	-A	0.124 (4)	0.0264 (8)	< 0.1	< 0.1	96.84
	-B	0.0750 (4)	0.0037 (60)	< 0.1	< 0.1	96.80

A - with iontophoresis.

B - without iontophoresis.

The units of k_p and t_L are in cm/hr and hrs respectively.

Table 6.2 Steady state flux ($\mu g\ cm^{-2}\ hr^{-1}$) for the permeation of weak electrolytes through the human stratum corneum using DM III and linear regression (LR).

pH*	Methotrexate		Salicylic acid		Aspirin		Lignocaine hydrochloride		Chlorpromazine hydrochloride		Chlorpheniramine maleate		Ephedrine hydrochloride		Pilocarpine hydrochloride	
	DM III	LR	DM III	LR	DM III	LR	DM III	LR	DM III	LR	DM III	LR	DM III	LR	DM III	LR
3.4[e]	0.0250	0.022	-	-	5.5	6.5	214.0	220.0	-	-	9.6	11.0	-	-	-	-
3.6[e]	0.028	0.031	14.720	12.55	-	-	-	-	4.62	5.50	-	-	68.6	65.0	1.78	1.70
5.0[e]	0.055	0.032	8.336	24.48	-	-	-	-	-	-	-	-	-	-	-	-
5.2[e]	-	-	-	-	27.6	14.0	213.6	216.4	4.62	5.20	9.6	10.0	-	-	-	-
5.8[e]	0.060	0.035	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6.6[e]	-	-	8.010	32.76	28.0	30.0	-	-	-	-	-	-	68.6	64.2	-	-
7.2[e]	-	-	8.000	34.50	28.0	31.0	184.2	186.2	0.46	0.47	9.6	9.8	68.6	61.0	-	-
8.0[e]	0.060	0.058	8.000	35.08	28.0	31.0	114.2	116.0	-	-	-	-	68.4	60.0	7.80	7.22
8.4[f]	-	-	-	-	-	-	-	-	0.48	0.50	9.0	8.0	-	-	-	-
9.4[f]	-	-	-	-	-	-	40.5	42.0	0.48	0.50	7.2	6.5	-	-	-	-
9.6[f]	-	-	-	-	-	-	-	-	-	-	-	-	65.5	66.0	-	-
11.7[f]	-	-	-	-	-	-	35.1	34.2	0.49	0.60	5.9	6.6	62.4	63.0	-	-

e and f are the symbols for the buffer systems shown in Table 3.2.

*pH measured at 25°C.

Fluxes in absence of iontophoresis are shown in Tables 5.3 and 5.6.

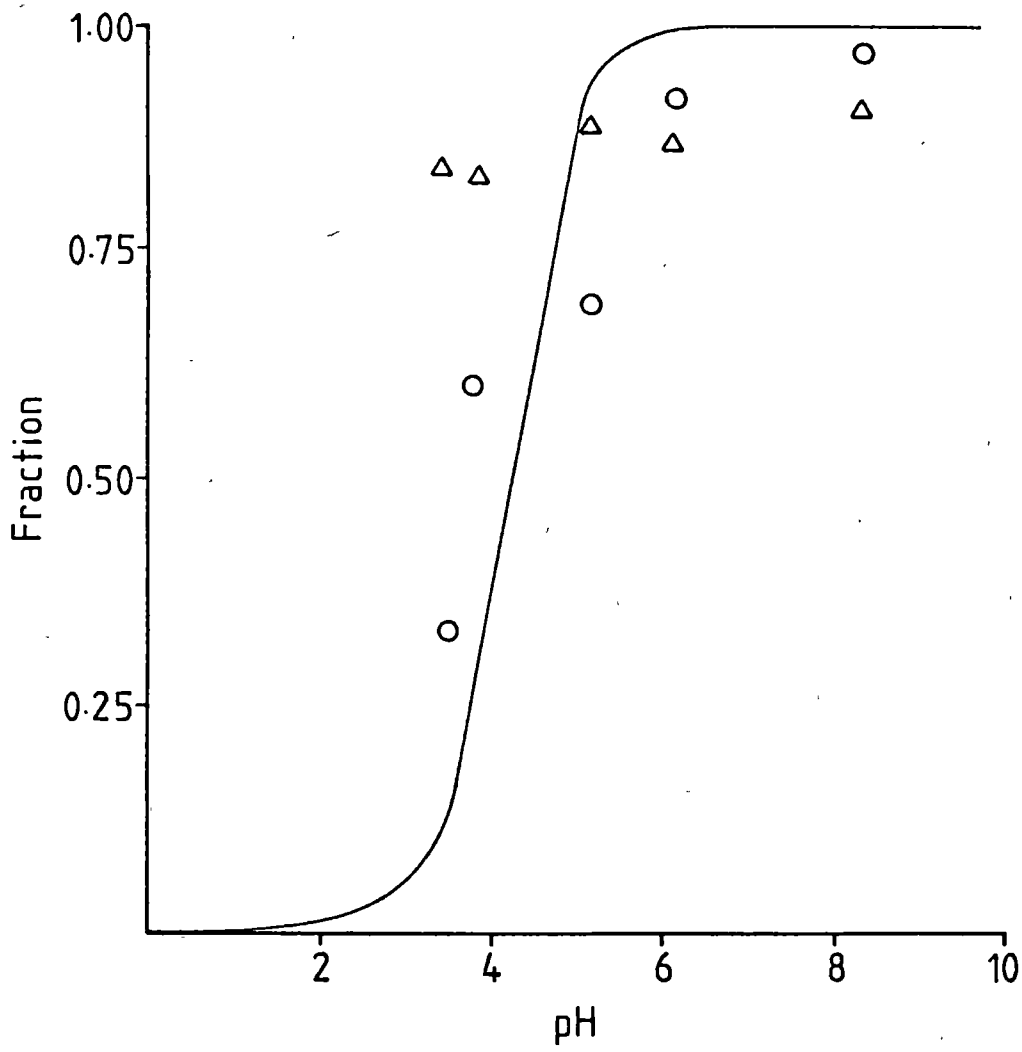


Figure 6.9 Effect of vehicle pH on the fraction change in the steady state flux for methotrexate (○) and tritiated water (△) through the human stratum corneum. The fraction of methotrexate ionised is indicated by the solid line.

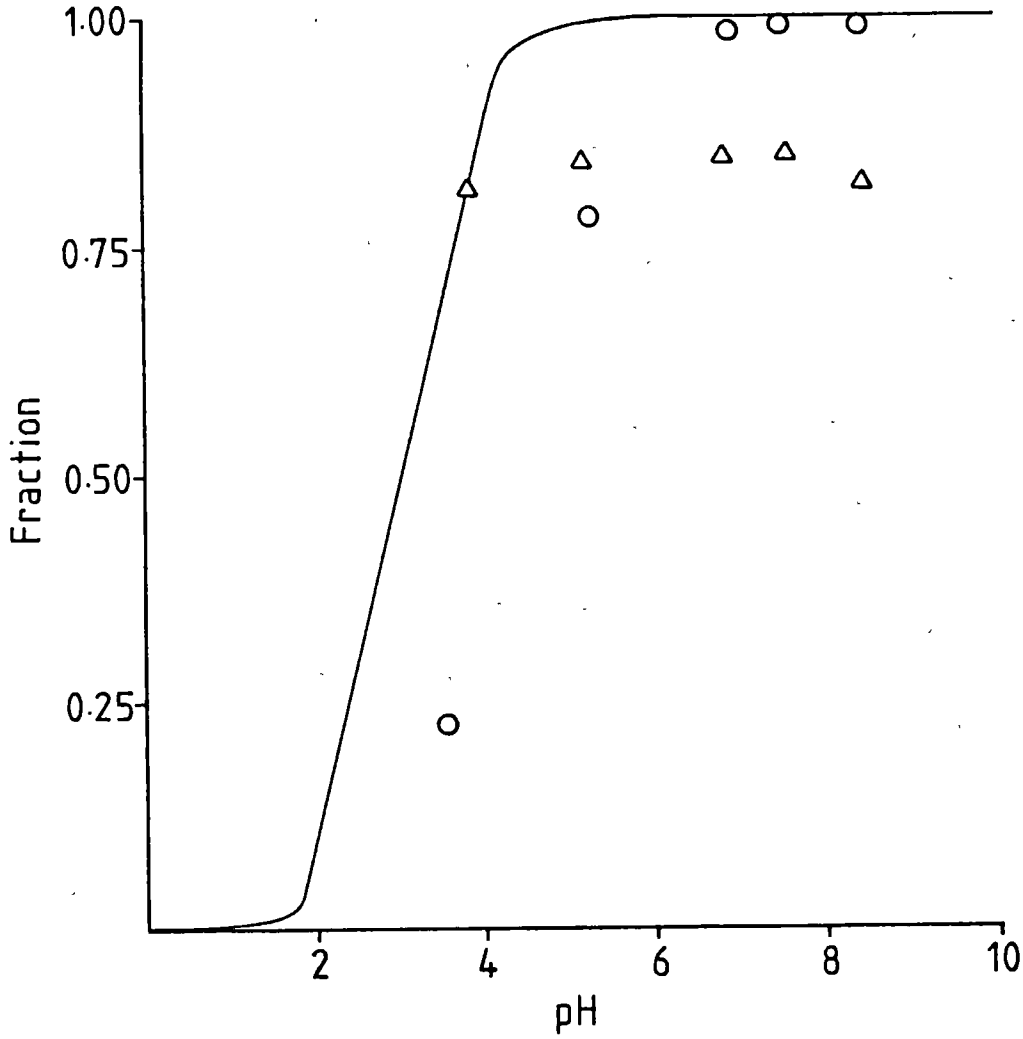


Figure 6.10 Effect of vehicle pH on the fraction change in the steady state flux for salicylic acid (○) and tritiated water (△) through the human stratum corneum. The fraction of salicylic acid ionised is indicated by the solid line.

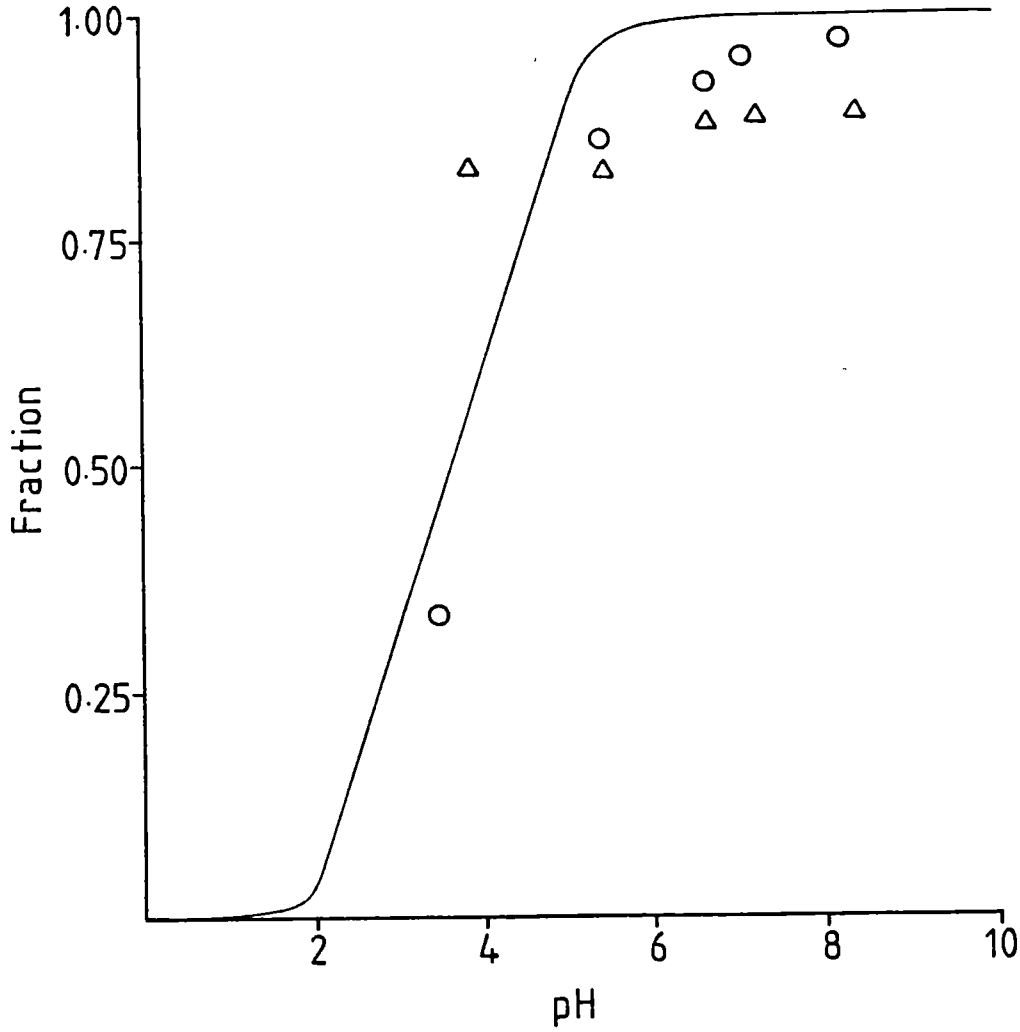


Figure 6.11 Effect of vehicle pH on the fraction change in the steady state flux for aspirin (○) and tritiated water (△) through the human stratum corneum. The fraction of aspirin ionised is indicated by the solid line.

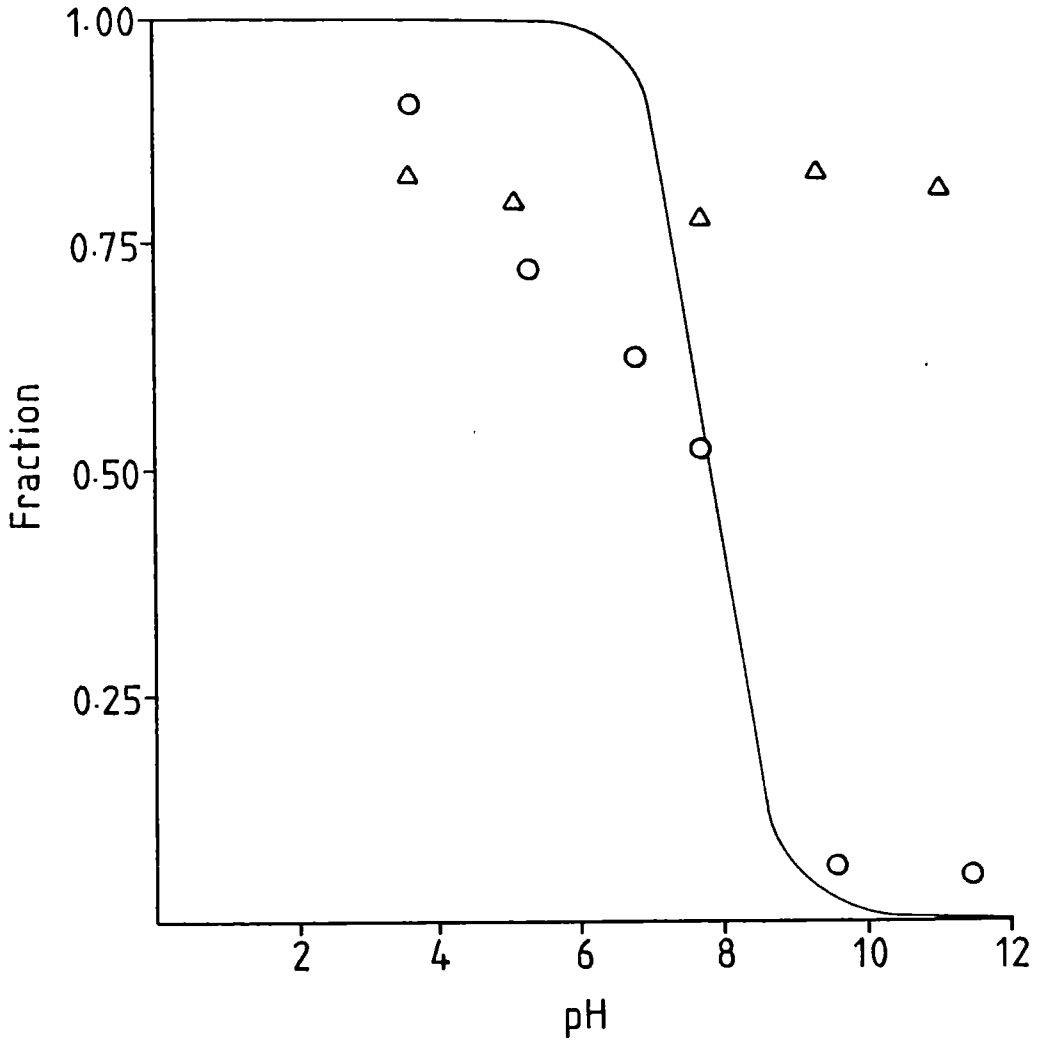


Figure 6.12 Effect of vehicle pH on the fraction change in the steady state flux for lignocaine hydrochloride (○) and tritiated water (△) through the human stratum corneum. The fraction of lignocaine hydrochloride ionised is indicated by the solid line.

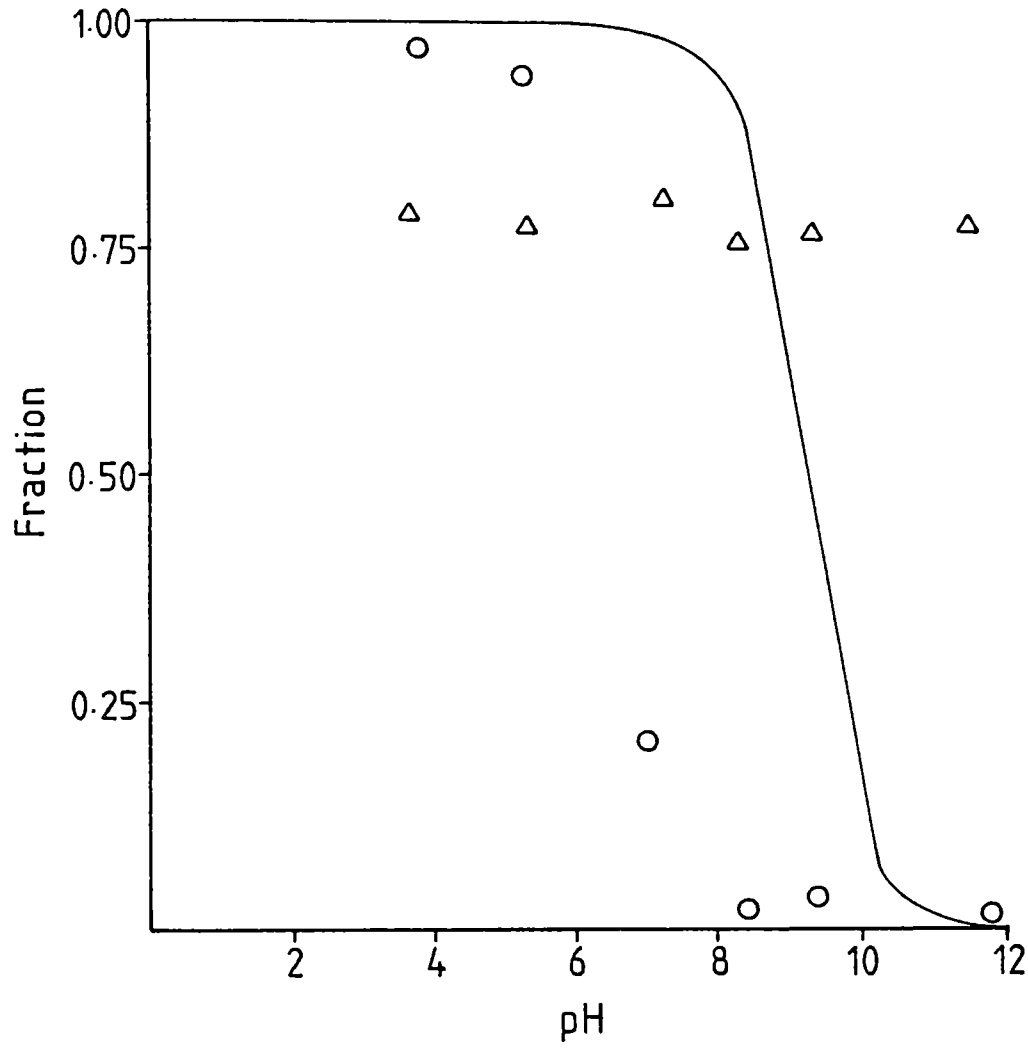


Figure 6.13 Effect of vehicle pH on the fraction change in the steady state flux for chlorpromazine hydrochloride (○) and tritiated water (△) through the human stratum corneum. The fraction of chlorpromazine hydrochloride ionised is indicated by the solid line.

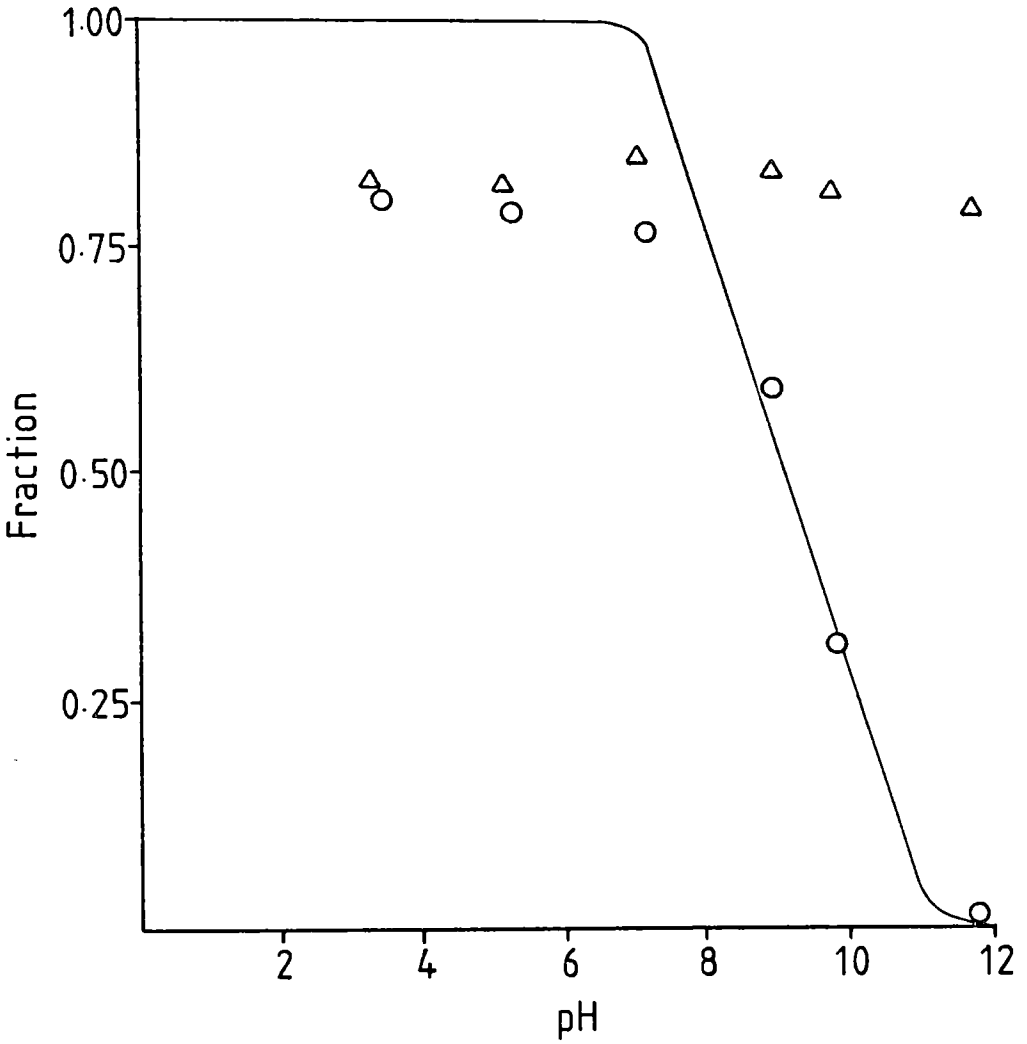


Figure 6.14 Effect of vehicle pH on the fraction change in the steady state flux for chlorpheniramine maleate (O) and tritiated water (Δ) through the human stratum corneum. The fraction of chlorpheniramine maleate ionised is indicated by the solid line.

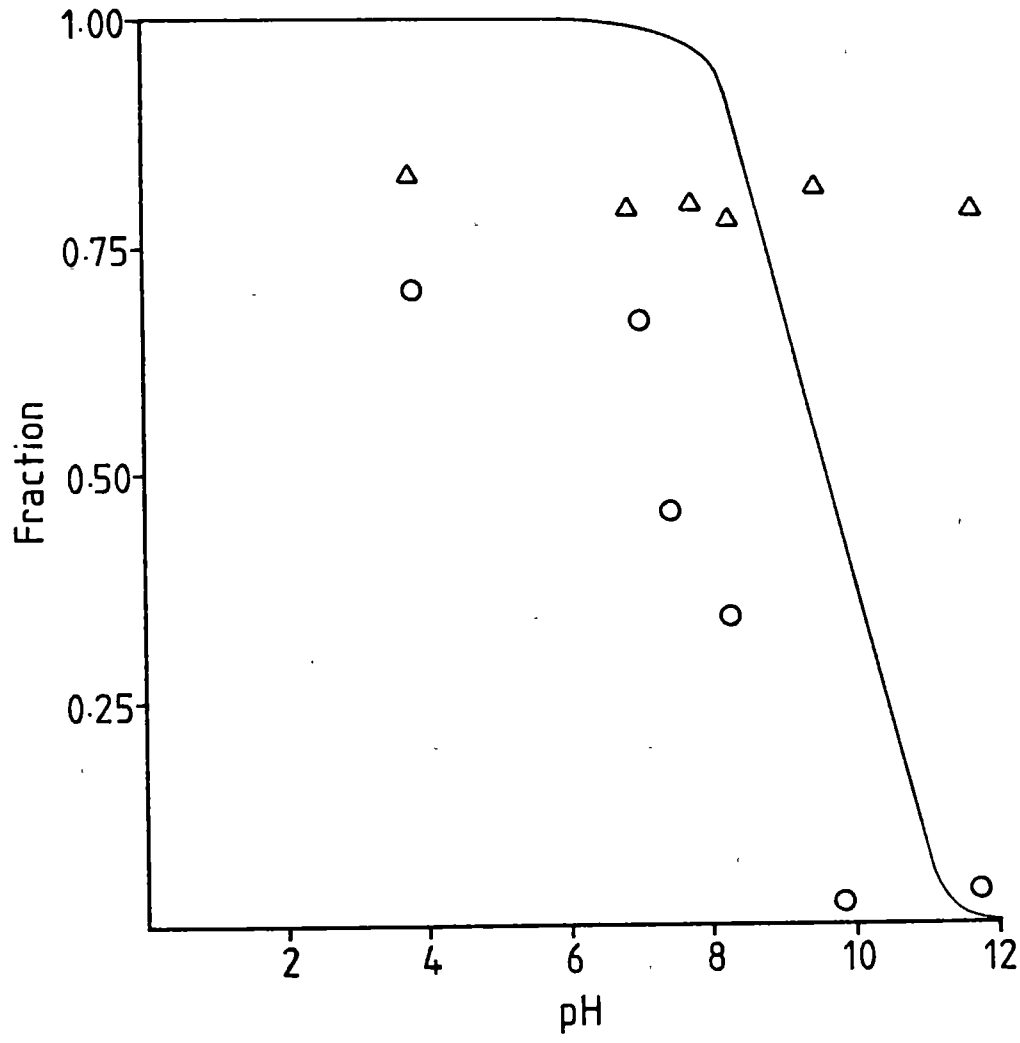


Figure 6.15 Effect of vehicle pH on the fraction change in the steady state flux for ephedrine hydrochloride (o) and tritiated water (Δ) through the human stratum corneum. The fraction of ephedrine hydrochloride ionised is indicated by the solid line.

of ionisation. As the extent of ionisation increases the flux, during application of iontophoresis, also increases. Examination of the data for different pH values reveals a significant difference (Appendix 5) between the fluxes with and without iontophoresis and a dependence on the degree of ionisation. A test of least significant difference (Appendix 5) also shows, at pH values at which there is a high degree of ionisation, evidence of significant differences in fluxes with and without iontophoresis (lsd, $P<0.05$) while at pH values at which there is a low degree of ionisation, the change in the magnitude of the fluxes due to iontophoresis is not significant (lsd, $P>0.05$). This statistical analysis shows that when weak electrolytes are in a highly unionised form, the effect of iontophoresis on the rate of penetration of the substance(s) is negligible (Appendix 5).

6.3 Discussion

Integrity of stratum corneum

The exposure of the stratum corneum to aqueous solutions of different pH values with and without iontophoresis did not have any effect on the permeability of tritiated water. The fraction change in flux for water was found to be independent of pH for the stratum corneum specimens which were exposed to solutions of different pH values (Figure 6.9 to 6.15).

Relationship between iontophoresis and degree of ionisation

Iontophoresis is the transfer of ions through the stratum corneum in the presence of an electric current (Harris 1967). The

relationship between the rate of permeation of weak electrolytes during iontophoresis and the degree of ionisation (Figures 6.9 to 6.15) may possibly be related to the conductivity of drugs in aqueous solutions (Gangarosa et al 1978). Iontophoresis was found to be more effective over a wider pH range for acidic weak electrolytes in comparison to basic weak electrolytes used during this work (Appendix 5). However, all the drugs had sufficient conductivity to show a significant difference in the rate of permeation during iontphoresis at a pH value which gave more than 25% ionisation of the substance in aqueous solution (Figures 6.9 to 6.15). Gangarosa et al (1978) also reported that the conductivity of lignocaine decreased at higher pH's (decrease in ionisation of the base) and that the hydrochloride salts of local anaesthetics conducted best at pH 5.0 where all local anaesthetic molecules exist in the positively charged form. Similarly methotrexate was reported to have the highest conductivity due to the presence of a glutamate residue with two negative centres.

Route of skin penetration

The dependence of route of penetration on lag times (sections 2.2.1, Chapters 4 and 5) could also be considered for the permeation of ionic species with iontophoresis. The general reduction in lag times during iontophoresis, especially visible for methotrexate (Figure 6.1) and pilocarpine (Figure 6.8), indicates that the transport of ionic species of weak electrolytes through excised human stratum corneum may occur via "shunts". However, the small fractional area of "shunts" and the effect of hydration on the excised human stratum corneum

(Chapters 4 and 5) would not support this hypothesis. Alternatively it may be possible that both routes of skin penetration ("intracellular" and "shunts") are responsible for the fast rate of penetration of weak electrolytes, with "shunts" being the dominant route of skin penetration during iontophoresis.

On the other hand it may also be possible, due to the application of the current during iontophoresis and the short duration of the experiments, that the lumens of the sweat ducts do not become swollen (Chapter 4 and Scheuplein et al 1969), and may act as "shunts" during the present in vitro experiments. Therefore it is more likely that the "shunt" pathway could be the dominant route of skin penetration during both in vitro and in vivo iontophoresis of lignocaine (Russo et al 1980) and pilocarpine (Gibson and Cooke 1959; Kopito and Shwachman 1969), as both the drugs have been reported to be more effective topically when applied with iontophoresis.

Clinical significance of iontophoresis

Clinically, a pH of 5 to 6 (pH of the epidermis 4.2-6.5; from Katz and Poulsen, 1971) would be suitable for lignocaine and pilocarpine solutions to be applied to the skin with iontophoresis as this pH range would give sufficient conductivity for iontophoresis to be effective and safe. It has been reported by Russo et al (1980) that lignocaine was effective at pH 6.0 when administered iontophoretically to human volunteers. Similarly pilocarpine administered at pH 5.0, will be more suitable to induce sweating (Table 6.2 and Figure 6.8).

Conclusions

This section of the work shows that the increased anaesthetic effect found for lignocaine (Russo et al 1980) and the effectiveness of pilocarpine to induce sweating during iontophoresis was pH dependent. It may be possible that a number of other weak electrolytes which show poor percutaneous absorption could also be administered by iontophoresis provided the drug was applied in a highly ionised form.

CHAPTER 7

Topical efficacy of drugs: role of dermal transport

The topical efficacy of any drug will depend upon the concentration that can be attained in the epidermis. For drugs used in psoriasis the site of action is in the malphigian layer (stratum spinosum and stratum germinativum). The concentration of any drug at this site is however dependent on the rate of its penetration through the stratum corneum and its clearance in the dermis (Figure 1.2). On the other hand for antifungal agents the site of action is in the stratum corneum (Figure 1.2) and the rate of penetration into the stratum corneum alone is important (Chapter 2).

Methotrexate has been shown to be effective in the management of psoriasis after oral or systemic administration (Schaefer et al 1982). In order to avoid potential systemic toxicity there has been considerable interest in the possibility of administering methotrexate topically (Comaish and Juhlin 1969; Wallace et al 1972; Weinstein 1977; Wantzin and Thomsen 1983). Most investigators agree however that topically applied methotrexate is ineffective (Weinstein 1977; Wallace et al 1978; Weinstein 1981; Ball et al 1982). Fry and Mcminn (1967) have however claimed that topical methotrexate is effective against psoriasis. Most studies quantifying the absorption rates of methotrexate through human skin have been limited to reporting epidermal permeability data obtained with intact human skin in vitro (Wallace et al 1978; Ball et al 1982).

The concentration of a steroid that can be attained in the epidermis will depend on the rate of its penetration through the

stratum corneum and its clearance in the dermis (Figure 1.2). The dermal clearance may be considerably reduced by vasoconstriction after the topical administration of steroids and the degree of vasoconstriction depends upon the potency and the concentration of the steroid at the site of the action (Schaefer et al 1982).

The present section describes the studies carried out to determine the relative importance of the epidermal and the dermal barriers in the topical effectiveness of methotrexate and steroids. The effect of the rate of perfusion on the in vitro rate of permeation of steroids through the human stratum corneum (Chapter 4) was also used to relate the significance of dermal clearance.

Theory

The kinetics of in vitro and in vivo disappearance of solutes into the dermis was assumed to be either monoexponential or biexponential in nature. Using the approach of Levy and Rowland (1974), this disappearance was examined using a two compartment open model in which compartment 1 represents the donor solution, compartment 2 the dermis and compartment 3 the blood and other fluids of distribution. The disappearance profiles of solutes can be represented by the biexponential equation which is written as:

$$F_s = A \exp^{-\alpha t} + (1-A) \exp^{-\beta t} \qquad \text{Eq. 7.1}$$

where F_s is the fraction of solute remaining in the dermal absorption cells, A and B are the fractional zero-time intercepts, alpha and beta are the fast and the slow rate

constants respectively.

The clearance (Cl) can be estimated by the following equation:

$$Cl = \text{donor volume} / \left(A/\alpha + \{(1-A)/\beta\}\right) \qquad \text{Eq. 7.2}$$

If A is very small and/or alpha is very large, it may not be possible to correctly estimate the initial (distribution) phase accurately (Levy and Rowland 1974). In this situation, the clearance can be approximately defined as:

$$Cl = \frac{\text{donor volume} \times k}{B} \qquad \text{Eq. 7.3}$$

and

$$V_{d_{ss}} = \frac{\text{donor volume}}{B} \qquad \text{Eq. 7.4}$$

where k is the pseudo first order rate constant and B is the intercept of the steady state portion of the line on the y-axis. In case of monoexponential disappearance (e.g. methotrexate) B is equal to one.

According to Levy and Rowland (1974) the volume of distribution at steady state (V_{dss}) is related to the volume of solution in the donor compartment (V_1), the volume of distribution in the dermis (V_2) and the partition coefficient between the dermis and the aqueous solution (R):

$$V_{d_{ss}} = V_1 + RV_2 \qquad \text{Eq. 7.5}$$

The apparent permeability coefficient (k_p) of methotrexate is calculated using equation 2.5. Since it has been shown in Chapter 5 that both ionised and unionised species of methotrexate

can permeate the excised human stratum corneum, C_v used is the constant total donor (unionised + ionised) concentration.

The steady state concentration (C_{ss}) likely to be achieved in the viable epidermis is estimated using the clearance into the dermis and the rate of permeation through the stratum corneum (Roberts et al 1982):

$$C_{ss} = \frac{k_p C_v A}{Cl} = \frac{J_{ss} A}{Cl} \qquad \text{Eq. 7.6}$$

where k_p is the permeability of the stratum corneum of area A, C_v is the concentration of the solute in the solution applied to the stratum corneum and Cl is the clearance of solute from from the viable epidermis. The minimum rate of input required (R_o) to achieve a given steady state concentration (C_{ss}) in the viable epidermis is obtained by rearranging equation 7.6:

$$R_o = C_{ss_r} Cl \qquad \text{Eq. 7.7}$$

7.1 Experimental

Permeation runs were carried out using the method described in sections 3.3.1 and 3.4, and the <u>in vitro</u> and <u>in vivo</u> dermal studies were carried out using the technique described in section 3.5. The method of analysis is shown in Table 3.1. Non-linear regression (unweighted) was carried out using equation 7.1 and individual data points (section 3.7.5). Pseudo first order rate constants and the intercept (B) was estimated by linear

regression.

7.2 Results

7.2.1 Methotrexate

Rate of disappearance into the dermis

Figure 7.1 shows the percentage of methotrexate remaining in solution following application to the human and rat dermis. A monoexponential decline was observed in these studies (B = 1). The rate of disappearance in rat dermis is comparable with that obtained for human dermis at 37°C. The rate of disappearance of methotrexate was found to depend on the volume of the solution applied, mean rate constants of 0.04 and 0.022 hr^{-1} being found for the disappearance of methotrexate into human dermis for 4 and 6 ml respectively. The corresponding values of clearance (equation 7.3) obtained for these volumes are 0.15 and 0.13 ml hr^{-1}, respectively, and are not significantly different ($P > 0.05$, t-test).

The clearance of methotrexate from the viable epidermis is also dependent on dermal blood supply. In anaesthetised rats, the clearance of methotrexate is higher than that obtained in dead animals (Figure 7.1). The clearances estimated from the data in Figure 7.1 are given in Table 7.1.

Rate of entry into the stratum corneum

Table 7.2 shows the steady state flux obtained for methotrexate applied to the stratum corneum. The flux is pH dependent; with increase in pH the flux was found to decrease (Chapter 5).

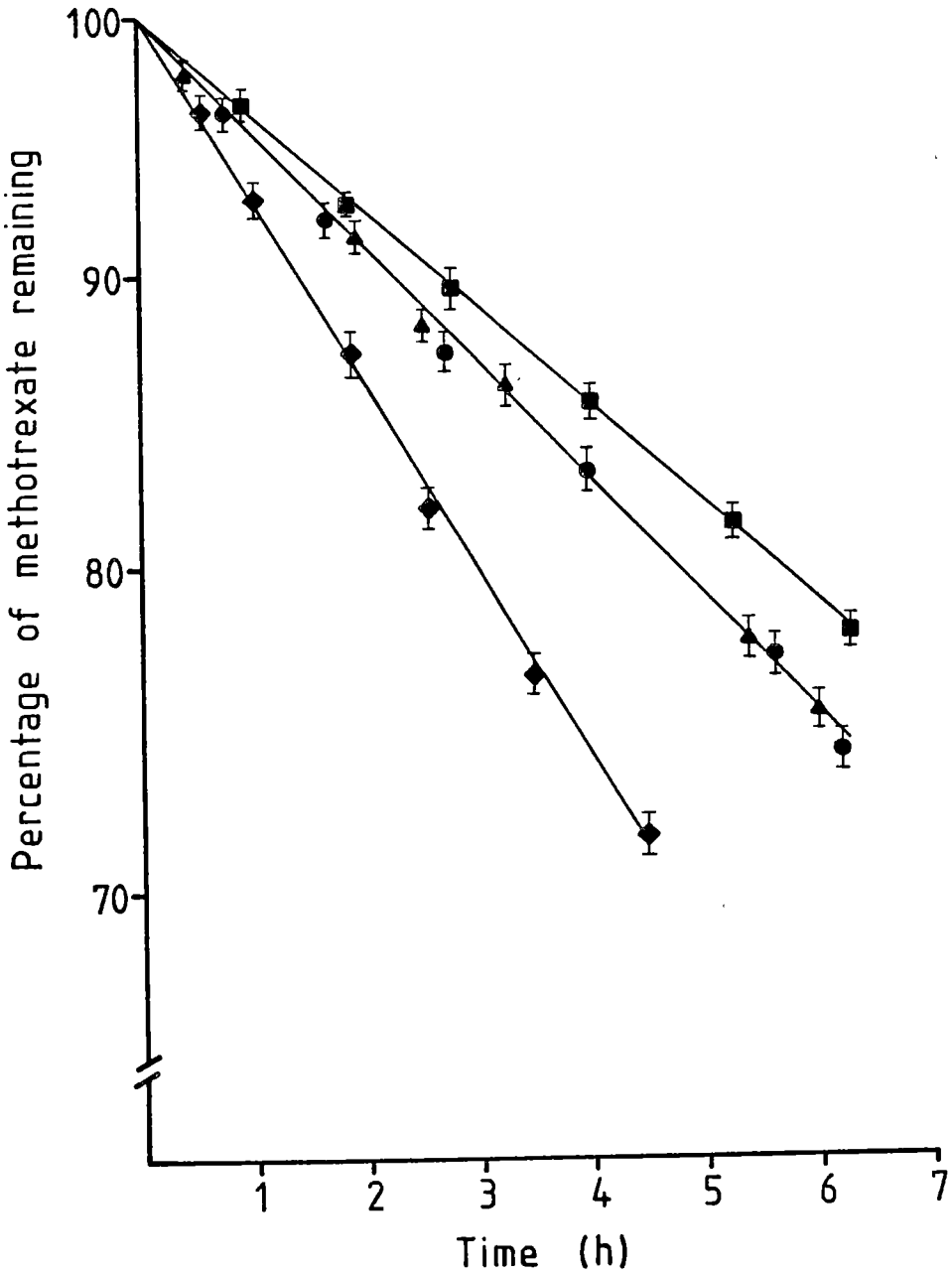


Figure 7.1 Disappearance of methotrexate into dermis (log scale). Mean ± SD, n=4. ■ Human dermis 25°C, ▲ human dermis 37°C, ● dermis of dead rat 37°C, ◆ dermis of anaesthetised rat 37°C.

Table 7.1 Clearance of methotrexate into the dermis from aqueous solutions containing 0.003% methotrexate.

Dermal preparation	Clearance[a] ml/hr
Human (25°C) postmortem	0.12 ± 0.011
Human (37°C) postmortem	0.15 ± 0.012
Rat (37°C) sacrificed	0.20 ± 0.011
Rat (37°C) anaesthetised	0.30 ± 0.013

[a] mean ± S.D. (n = 4).

Table 7.2 Effect of pH on the permeation of methotrexate through human stratum corneum at 25 ± 1°C for aqueous solutions containing 0.003% methotrexate.

pH (25°C)	Number of runs	steady state flux (Jss)* $ng/cm^2/hr$	concentration in stratum corneum at steady state ng/mg
3.4[a]	2	13.0 (12.6,13.5)	190 ± 10
3.6[b]	2	11.0 (10.0, 11.7)	410 ± 16
5.0[a]	2	10.0 (10.1, 9.8)	500 ± 10
5.8[c]	3	8.8 (8.6,8.8,9.0)	60 ± 10
8.0[a]	4	2.2 (1.9,2.3,2.4,2.0)	50 ± 5

a, b and c are for different buffers shown in Table 3.2.

* The numbers in parenthesis represent individual Jss values.

Larger concentrations of residual methotrexate were found at pH values at and below 5.0. In this work the effect of receptor composition on the permeation of methotrexate through human stratum corneum was also evaluated. The rate of penetration was independent of the type of receptor fluid used. The flux for 0.003% methotrexate (pH 5.8) was 8.75 ng cm^{-2} hr^{-1} for isotonic sodium chloride and 8.90 ng cm^{-2} hr^{-1} for an aqueous protein solution (SPPS). In all studies, the cumulative percentage recovered in the receptor solution was less than 5%.

Concentrations in the viable epidermis

Table 7.3 shows the apparent unbound steady state concentrations estimated for the viable epidermis. These values are derived from dermal absorption (Table 7.1) and the stratum corneum permeability (equation 2.5) calculated from values of flux (Table 7.2), the unbound steady state concentrations range between 0.5 to 0.03 ug ml^{-1}. Higher concentrations are observed for acidic solutions applied to skin in which no blood supply exits (Table 7.3).

7.2.2 Steroids

Rate of disappearance into the dermis

Figure 7.2 shows the percentages of steroids remaining in the solution following application to human and rat dermis. The rate of disappearance in the sacrificed rat dermis was of similar magnitude to that obtained for human dermis at 37°C, while the rate of disappearance of steroids in the anaesthetised rat dermis was found to be higher than that obtained in the dead animals.

Table 7.3 Estimated steady state concentration (C_{ss}) in the viable epidermis calculated using the data given in Tables 7.1 and 7.2

pH of aqueous solution applied to stratum corneum	Dermal preparations			
	human 25°C postmortem	human 37°C postmortem	rat 37°C sacrificed	rat 37°C anaesthetised
		µg/ml		
3.4[a]	0.49	0.39	0.30	0.20
3.6[b]	0.45	0.36	0.27	0.18
5.0[a]	0.38	0.30	0.23	0.15
5.8[c]	0.35	0.28	0.21	0.14
8.0[a]	0.10	0.06	0.05	0.03

a, b and c are for different buffers shown in Table 3.2

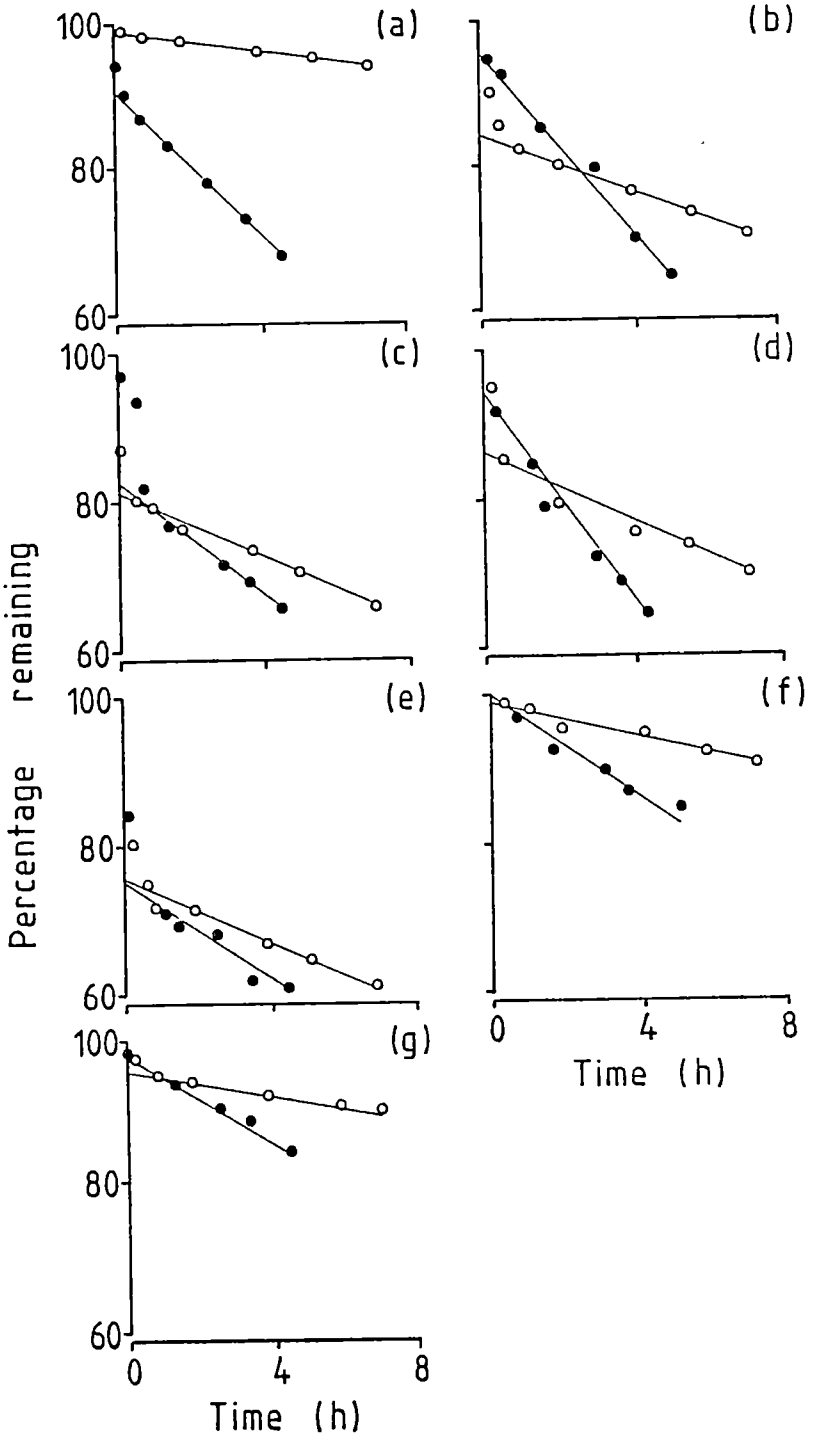


Figure 7.2 Disappearance of steroids into dermis. (a) Triamcinolone; (b) Hydrocortisone; (c) Prednisolone; (d) Corticosterone; (e) Tiamicinolone acetonide; (f) Testosterone; (g) Betamethasone 17-valerate. O Human and rat dermis, ● anaesthetised rat dermis.

Table 7.4 shows the clearance estimated using equation 7.3. The clearance was found to be higher for anaesthetised rat dermis, indicating the importance of dermal blood supply.

Table 7.5 shows the apparent volume of distribution of steroids for the human and rat dermis. The volume of distribution was found to be slightly higher for the rat dermis in comparison to human dermis. However, there was no significant difference between volume of distribution estimated for the dermis of the sacrificed or the anaesthetised rats for each steroid ($P < 0.05$, t-test).

Concentrations in the viable epidermis

Table 7.6 shows the apparent unbound steady state concentration in the viable epidermis estimated from the clearance values of Table 7.4. The concentrations in the viable epidermis were also found to be higher in the absence of normal blood supply, and with the increase in the rate of input there was also an increase in the apparent concentration of steroids likely to be attained in the viable epidermis.

Importance of octanol/water partition coefficients

Figure 7.3 shows the relationship between clearance and octanol/water partition coefficients. The clearance was generally found to decrease with increase in partition coefficient. However, the polarity of steroids did not had any marked effect on the volume of distribution for the animal or human dermal preparations (Table 7.5).

Table 7.4 Clearance of aqueous solutions of steroids (pH 7.2) into the dermis at 37°C

Steroids	Clearance[a] ($ml\ hr^{-1}$)		
	Human dermis[b]	Sacrificed rat dermis[b]	Anaesthetised rat dermis
Triamcinolone (0.0024%)	0.072 ± 0.002	0.082 ± 0.004	0.383 ± 0.030
Hydrocortisone (0.003%)	0.100 ± 0.005	0.112 ± 0.003	0.361 ± 0.040
Prednisolone (0.0035%)	0.086 ± 0.003	0.100 ± 0.005	0.220 ± 0.004
Corticosterone (0.0024%)	0.031 ± 0.001	0.037 ± 0.002	0.184 ± 0.006
Triamcinolone acetonide (0.0024%)	0.087 ± 0.004	0.102 ± 0.002	0.211 ± 0.005
Testosterone (0.002%)	0.031 ± 0.005	0.036 ± 0.006	0.142 ± 0.005
Betamethasone 17-valerate (0.001%)	0.018 ± 0.002	0.022 ± 0.001	0.094 ± 0.008

a - Mean ± SD, n = 3.
b - pseudo first order rate constants were of similar magnitude for both the dermal preparations.

Table 7.5 Apparent volume of distribution (V_{dss}) of steriods (pH 7.2) into the dermis at 37°C.
Volume of donor compartment for human and animal dermal studies was 3 and 4 ml respectively).

Steroids	Apparent volume of distribution (ml)[a]		
	Human dermis[b]	Sacrificed rat dermis[b]	Anaesthetised rat dermis
Triamcinolone (0.0024%)	3.07 ± 0.12	4.09 ± 0.28	4.37 ± 0.26
Hydrocortisone (0.003%)	3.37 ± 0.42	4.49 ± 0.45	4.81 ± 0.78
Prednisolone (0.0035%)	3.61 ± 0.22	4.81 ± 0.24	4.88 ± 0.23
Corticosterone (0.0024%)	3.14 ± 0.26	4.19 ± 0.22	4.59 ± 0.25
Triamcinolone acetonide (0.0024%)	3.96 ± 0.42	5.27 ± 0.28	5.30 ± 0.30
Testosterone (0.002%)	3.05 ± 0.19	4.07 ± 0.22	4.12 ± 0.21
Betamethasone 17-valerate (0.001%)	3.07 ± 0.25	4.09 ± 0.21	4.20 ± 0.22

a - Mean ± SD, n = 3.

b - Intercept (B) was of similar magnitude for both the dermal preparations.

Table 7.6 Estimated steady state concentration (C_{ss}) of steroids in the viable epidermis using the data given in Tables 4.3 and 7.4.

Steroids	Dermal preparations C_{ss} ($\mu g\ ml^{-1}$)								
	human-postmortem			rat-sacrificed			rat-anaesthetised		
	0 ml hr^{-1}	10 ml hr^{-1}	20 ml hr^{-1}	0 ml hr^{-1}	10 ml hr^{-1}	20 ml hr^{-1}	0 ml hr^{-1}	10 ml hr^{-1}	20 ml hr^{-1}
Triamcinolone	0.0059	0.0096	0.0099	0.0052	0.0084	0.0087	0.0011	0.0018	0.0019
Hydrocortisone	0.0038	0.0081	0.0101	0.0034	0.0072	0.0090	0.0011	0.0022	0.0028
Prednisolone	0.0818	0.0879	0.0916	0.0704	0.0756	0.0788	0.0320	0.0344	0.0358
Corticosterone	0.0781	0.0836	0.1000	0.0654	0.0701	0.0847	0.0131	0.0141	0.0170
Triamcinolone acetonide	0.0251	0.0170	0.0243	0.0214	0.0145	0.0208	0.0103	0.0070	0.0100
Testosterone	2.4711	2.6129	2.7871	2.1279	2.2500	2.4000	0.5395	0.5704	0.6085
Betamethasone 17-valerate	2.7880	3.4239	4.0353	2.3482	2.8636	3.3750	0.5496	0.6702	0.7899

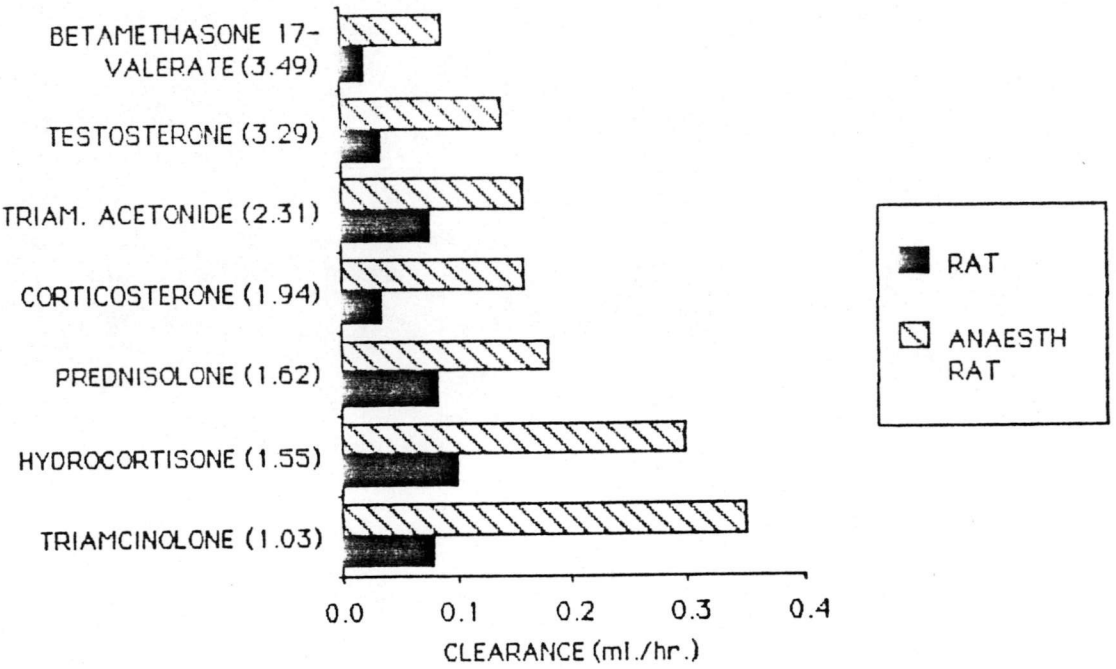


Figure 7.3 Relationship between octanol/water partition coefficient and clearance.

7.3 Discussion

7.3.1 Methotrexate

Reasons for the failure of topical therapy

It has been suggested that the failure of topical methotrexate in psoriasis results either from insufficient percutaneous penetration (Wallace et al 1978) or because methotrexate might influence a factor in other organs which is responsible for the control of psoriasis (Stewart et al 1972). It has also been reported that inhibition of polymorphonuclear leucocyte activity (Walsdorfer et al 1983) is also important and there is a possibility that the substance passes very rapidly through the skin thereby preventing the attainment of a concentration sufficient to inhibit the epidermal DNA synthesis necessary to maintain psoriasis (Schaefer et al 1982).

Importance of dermal clearance

The mechanism for the dermal clearance of any drug in the absence of vasoconstriction, appears to relate to two processes, (a) diffusion in the dermis and (b) removal in the blood perfusing in the dermis (Figure 1.2).

There is a negligible distribution of methotrexate between the solution in the donor compartment and the human or rat dermis (B = 1, Figure 7.1). This indicates that the fast removal of methotrexate into the dermis occurs from the donor compartment mainly due to diffusion and removal by the blood perfusing the

dermis.

In the pharmacokinetic approach proposed by Rowland and Tozer (1980), clearance and volume are independent variables. According to this approach, the unbound concentration of a drug in the viable epidermis (Figures 1.1 and 1.2) is only dependent on the rate of drug delivery to the site and the clearance from this site. The total concentration of a drug is determined not only by these parameters but also by the binding of the drug to other components in the viable epidermis (Schaefer et al 1982).

The clearance of methotrexate from the aqueous solution into the human dermis is relatively unaffected by temperature (Table 7.1). In contrast, the transport of a number of compounds through the stratum corneum (in vivo) has been shown to be temperature dependent (Table 2.4).

The clearance of methotrexate into the dermis was higher for the rat dermis than the human dermis (Table 7.1). An important factor controlling dermal clearance was the dermal blood supply, the clearance of methotrexate being 50% higher in the anaesthetised rats in comparison to dead rats (Figure 7.1, Table 7.1). The use of anaesthetised and dead rats to demonstrate the role of blood supply in drug absorption has been previously used by Levy and Rowland (1974). These workers reported a biphasic disappearance profile for local anaesthetics applied to subcutaneous tissue and it was proposed that lignocaine derivatives partition into the subcutaneous tissue with a subsequent loss to the blood stream. A biphasic profile is most likely when significant accumulation in the dermis/subcutaneous tissue occurs. As dermis is predominantly

aqueous (Chapter 2) and methotrexate is a water soluble drug (McCullough et al 1976), therefore a significant accumulation of methotrexate after application as aqueous solution is not likely and hence a monoexponential profile (Figure 7.1). It is more likely that methotrexate is irreversibly lost from the site, after topical application, at a very fast rate.

Abolition of dermal supply only partly reduces dermal clearance, suggesting that the clearance of methotrexate consists of both the diffusion in the dermis and the parallel removal by the dermal blood supply (Figure 1.2). Methotrexate was quantified in the receptor compartment in human dermal studies, the rate constant for appearance (0.0282 hr^{-1}) at early times being less than the rate constant for disappearance (0.04 hr^{-1}). The slow rate of appearance is consistent with the transport being diffusion controlled (Figure 1.2).

Prediction of concentration in the viable epidermis

A minimal theoretical concentration of less than 1 microgram ml^{-1} for methotrexate in the viable epidermis has been suggested as being required for effective management of psoriasis (Schaefer et al 1982). The estimated concentrations given in Table 7.3 are lower than this value.

According to Equation 7.7 a flux of about 60 microgram cm^{-2} hr^{-1} is required from the stratum corneum to achieve the minimum effective concentration of methotrexate in the viable epidermis. This flux is about five times that observed in this study where aqueous solutions were applied to isolated stratum corneum at

25°C. In psoriasis the structural alterations in the stratum corneum facilitate faster permeation and the dermis also undergoes morphological changes (Handley et al 1983) with a possible increase in clearance.

High concentrations of methotrexate (50-510 ng ml^{-1}) were found in the stratum corneum after topical application (Table 7.2). As these concentrations are much higher than the minimal theoretical concentration required in the viable epidermis, it appears that removal of methotrexate by the dermis rather than penetration into the stratum corneum is more important in determining the efficacy of topical methotrexate. The data obtained for the pH dependence of epidermal penetration (Table 7.2) is consistent with results reported previously for full thickness hairless mouse skin (Wallace et al 1978, section 5.2.1).

Enhancement of percutaneous penetration

Considerably enhanced penetration may be achieved with an occlusive dressing which (a) raises the temperature of the stratum corneum and (b) promotes hydration of the stratum corneum (section 2.3.1.2). The other possible way of enhancing percutaneous penetration is by the reduction of dermal clearance by use of vasoconstrictors and adjustment of pH of the formulation applied to the skin (Table 7.3).

7.3.2 Steroids

Mechanism of dermal clearance

The mechanism for dermal clearance of steroids appears to relate

to three processes, (a) the occurence of vasoconstriction in the skin, (b) diffusion in the dermis and (c) removal in the blood perfusing the dermis. The visual examination of the dermis of anaesthetised rats did not reveal any blanching as the concentrations of steroids used during this work were too low to cause any vasoconstriction within the dermis (Schaefer et al 1982).

Kinetics of dermal disappearance

The diffusion of steroids in the dermis appears to follow a biphasic disappearance phenomenon which could be due to the accumulation of steroids in the predominantly aqueous environment of the dermis and also removal in the receptor compartment. Similar conclusion were drawn by Levy and Rowland (1974) for the accumulation of lignocaine in the subcutaneous tissues. The disappearance data (Figure 7.2) indicates that a rapid and significant distribution of steroids occurs between the solution in the dermal cell and the dermis, followed by a slower transfer of steroids from the donor compartment.

As the V_{dss} (equation 7.4) for all steroids is only slightly greater than the volume in the donor compartment (Table 7.5), only a limited amount of steroid appears to be present in the dermis due to distribution.

The initial phase for the loss of steroids from the cell is very rapid in comparison to the terminal phase (Figure 7.2). This indicates that the steroids equilibrate rapidly after initial contact with the dermis after the fractional rate of loss for

individual steroids becomes equal. This also implies that the initial loss occurs mainly due to partitioning of the steriods into the dermis and removal later by the receptor compartment (blood, body fluids or the receptor medium). The smaller rate constant for appearance in the receptor compartment (e.g. 0.022 hr^{-1} - hydrocortisone) relative to the disappearance from the donor compartment (e.g. 0.030 hr^{-1} - hydrocortisone) is consistent with slow accumulation of steroids occuring in the dermis. Similar rate constants were observed for the non-polar steroids.

The two compartment open model did not adequately described the data obtained during the present work. The reason for the discrepancy could be due to the absence of sufficient number of data points in the early stages of dermal disappearance of steroids which effected the correct estimation of rate constants. It may also be possible that the dermis acts as a diffusion barrier and the transport of solutes through it is diffusion controlled. A diffusion model to describe the transport of steroids through the dermis was not developed.

Significance of aqueous dermal environment and blood flow

It was found that as the polarity decreases the dermal clearance also decreases. The dermal clearance was also found to increase in the presence of normal blood supply (Figure 7.3). The apparent volume of distribution in the dermis was found to be independent of the polar nature of steroids.

The fast rate of disappearance in the subsequent phase of dermal

studies can be clearly seen in Figure 7.2. The similarities in the early disappearance could therefore be an indication that the initial distribution of steroid(s) between the donor compartment and the dermis was not dependent upon dermal blood supply, whereas subsequent removal of steroids from the site was dependent upon blood flow. The close similarities of the initial disappearance rates from the anaesthetised and postmortem dermis also indicates the absence of vasoconstriction.

Prediction of concentration in the viable epidermis

The estimated unbound steady state concentrations (C_{ss}) of steroids in the viable epidermis (Table 7.6) were generally found to increase with the increase in the input and the clearance of steroids in the dermis (Tables 4.3 and 7.4). The blood flow in the skin is approximately 0.05 ml/min per cubic centimeters at 23°C (Flynn 1979), thus for the permeation cell shown in Figure 3.2, a rate of *in vitro* perfusion of 10 ml hr^{-1} might mimic *in vivo* conditions. Therefore using the *in vitro* permeabiltiy coefficients obtained at 10 ml hr^{-1} (Table 4.3) and dermal clearance in anaesthetised rats (Table 7.4), it might be possible to estimate the concentrations that can be achieved in the viable epidermis provided there is no vasoconstriction. However, in the presence of vasoconstriction much higher concentrations, similar to human or rat dermal preparations, can be obtained.

Conclusions

The results obtained in this section of the work suggest that the rate of the loss into the dermis may be an important factor in

determining the overall rate of disappearance of methotrexate following topical application. The results reported here support the hypothesis (Schaefer et al 1982) that it is not the inability of methotrexate to permeate the stratum corneum which is the reason for its topical ineffectiveness even at high pH. The present data suggests that methotrexate is removed rapidly after passing through the stratum corneum, thereby preventing the attainment of a sufficient concentration in the malphigian layer to inhibit the epidermal DNA synthesis necessary to maintain psoriasis.

The results obtained in this section of the work suggest that the rate of blood flow in the absence of vasoconstriction, may determine the concentration of the steroid that can be attained in the viable epidermis. The high partition coefficient of a steroid may also reduce the overall clearance, thus enhancing the amount of the substance retained at the site of a disorder (Figure 1.2).

CHAPTER 8

CONCLUSIONS

Mechanism and route of skin penetration

It has been shown in the present work that the *in vitro* penetration of steroids and weak electrolytes through the excised human abdominal stratum corneum is more likely to be via the "intracellular" route rather than through the "shunts". This hypothesis is further substantiated by the scarcity of hair follicles in the abdominal area of the human body. In addition the possible swelling and deformation of excised stratum corneum (Scheuplein et al 1969), due to the hydration effect of the receptor fluid, may allow the penetration of steroids and highly ionised weak electrolytes through the "intracellular" route only.

There is substantial evidence, based on the use of mathematical models during the present work, to indicate that both the ionised and unionised species of a number of weak electrolytes are able to penetrate the excised human stratum corneum with relative ease (Chapter 5). Similar results for the gastrointestinal membrane were reported by the other workers (sections 1.2 and 1.5.1). The formation of an ion-pair and its ability to partition into the lipid phase with relative ease (Higuchi 1971), also suggests that the ionised species is likely to penetrate the excised human stratum corneum via the "intracellular" route rather than through the "shunts" (Chapter 5).

Although it has been known that individual ions or electrolytes can penetrate the skin (Tregear 1966a; Schaefer et al 1982), there is no physical evidence regarding the significance of

"shunts" during *in vitro* or *in vivo* transport of ions and/or ionised species through the human or animal skin (Chapters 1 and 2). Wahlberg (1968a) and Middleton (1969) have suggested that electrolytes are more likely to penetrate via the "intracellular" route. Scheuplein et al (1969) have suggested that long lag times, for more polar steroids (e.g. hydrocortisone; Chapter 4), indicate that the *in vitro* transport of these steroids through the "shunts" is important not only in the transient period (non-steady state penetration) but during the steady state penetration as well.

The mathematical models approach (Chapter 4) used during the present work does not support the above hypothesis of Scheuplein et al (1969) for the permeation of polar steroids through the "shunts". However, the permeation of non-polar steroids through the excised human stratum corneum via the "intracellular" route (Scheuplein et al 1969) is in agreement with the results found during the present investigations. Similarly the mathematical models and the evidence of the formation of ion-pairs of ionised weak electrolytes and nature of the skin (i.e. the effect of hydration and the scarcity of hair follicles on the abdominal skin, as discussed in Chapters 4 and 5), also indicate that the ionised species of weak electrolytes are more likely to permeate the excised human stratum corneum via the "intracellular" route. The transport of all the substances generally showed a faster rate of permeation through the inert membrane (devoid of "shunts") relative to the rate of permeation through the human stratum corneum (Chapters 4 and 5). This also suggests that there is a negligible contribution of "shunts" to the overall

permeation of either steroids (polar and non-polar) or weak electrolytes through the excised human abdominal stratum corneum.

Iontophoresis and mechanism of skin penetration

The in vitro iontophoresis experiments were carried out to determine the effect of pH on the rate of in vitro permeation of weak electrolytes through human stratum corneum. The results suggest that weak electrolytes penetrate faster with iontophoresis than without iontophoresis (Chapters 5 and 6), especially when the substance is in a highly ionised form.

The general reduction in lag times during iontophoresis (Chapter 6) indicates that the transport of ionic species of weak electrolytes through the excised human stratum corneum may occur via the "shunts". This is contrary to the above hypothesis, that is the mechanism of penetration weak electrolytes in the absence of iontophoresis, is likely to be through the "intracellular" route. It is possible however that both "shunts" and the "intracellular" route operate during the topical administration of drugs with iontophoresis (Chapter 6).

The short duration of in vitro iontophoresis experiments may not have permitted enough hydration of the excised human stratum corneum to cause swelling of the lumens of the sweat ducts. In addition the presence of facilitated transport, due to the application of current, may have allowed the "shunt" pathway to remain operative during the iontophoresis experiments. This hypothesis could be validated by the use of iontophoresis for the topical administration of lignocaine for local anaesthetic effect

(Russo et al 1980) and pilocarpine to induce sweating (Chapter 6). The site of topical administration of these drugs is usually on the forearm where the lumens of the sweat ducts are probably the dominant "shunt" pathway with a small contribution by the hair follicles. Due to the complex interdigitation, or interlocking of the cells by finger like processes, of the cells of the stratum corneum, any contribution by the "intercellular" route would be expected to be negligible (Blank and Scheuplein 1969). It may be possible, therefore, that both routes of skin penetration may be responsible for the fast rate of *in vitro* and *in vivo* penetration of weak electrolytes, with sweat ducts and hair follicles being the dominant route, through the stratum corneum during iontophoresis (Chapter 6).

Importance of perfusion in epidermal and dermal transport

The results obtained for the dermal clearance studies using methotrexate and steroids suggest that the rate of loss into the dermis, in the absence of vasoconstriction, may be an important factor in determining the overall rate of disappearance of these substances following topical application. The rate of blood flow in the absence of vasoconstriction, may determine the concentration of steroid(s) or methotrexate that can be attained in the viable epidermis (Chapter 7). The general increase in the rate of epidermal permeation of steroids through excised human stratum corneum with increase in the *in vitro* rate of perfusion (Chapter 4) can also be considered as an indication of the importance of blood flow and clearance in the dermis.

The *in vitro* rate of perfusion was found to become less

significant with increase in polarity of steroids or the increase in the epidermal permeability coefficients (Chapter 4). However, the dermal permeability coefficients of the steroids did not appear to depend on octanol/water partition coefficients, while the dermal clearance was found to decrease with decrease in polarity (Chapter 7). The topical efficacy of any drug depends on sufficient concentration being attained at the site of the disorder (Figure 1.2). Therefore to cure a disorder located in the viable epidermis it is important to determine the rate of epidermal penetration of a drug through the undamaged stratum corneum and its rate of removal by the dermis and the blood supply of the skin.

Topical therapy using methotrexate is ineffective mainly due to its fast removal in the dermis (section 7.3.1), while most of the steroids are topically effective as high concentrations can be attained in the viable epidermis even in the absence of vasoconstriction. Concentrations in the viable epidermis can be further enhanced due to vasoconstriction following topical administration of high concentrations of steroids (section 7.3.2, Schaefer et al 1982).

Importance of molecular weight and molecular structure

The penetration of steroids and weak electrolytes does not show any relationship between molecular weight and permeability coefficient (Tables 3.1, 4.2 and 6.1-k_p values in absence of iontophoresis). These results are in agreement with the previous reports that up to a molecular weight of at least 500, probably up to 5000 daltons, the molecular size plays no role in the rate

of percutaneous absorption of drugs (Schaefer et al 1982; section 2.3.1.6).

The results in the present work and those reported previously clearly show that the relative solubility of a substance in the lipid and aqueous phases of a system is likely to determine the extent of passive absorption of a substance through the skin (Chapters 1, 2 and 4).

Detailed structure activity relationship studies (Schaefer at al 1982; section 2.3.1.6) were not carried out during the present work. However, one relevant comparison which can be made is that between the rate of permeation of the two glucocorticoids, hydrocortisone and betamethasone 17-valerate. The change in the structure of the glucocorticoid resulted in the latter being therapeutically more effective than hydrocortisone (Flynn 1979). The main reason for the topical effectiveness of betamethasone 17-valerate over hydrocortisone could be due to the decrease in its polarity which results in increase in the rate of epidermal penetration and attainment of high concentrations in the viable epidermis (Chapters 1, 2, 4 and 7).

Strategies to control and optimize percutaneous absorption

In vitro methods are an important means of assessing various aspects of the percutaneous absorption of a drug. A good qualitative and even quantitative correlation can be made between *in vitro* and *in vivo* results (section 2.4). Therefore the *in vitro* results obtained in the present work could be used to develop various strategies to optimize and promote percutaneous

penetration of weak electrolytes and non-electrolytes (e.g. steroids) for either local or systemic effect.

It has been shown that it is the polarity of a substance which determines its rate of penetration through the human stratum corneum and that the rate of removal of the substance at the dermo-epidermal junction by diffusion into the dermis and blood vessels is important (Chapters 4 and 7). It follows that a steroid could be made therapeutically more effective by altering its structure to reduce its aqueous solubility, which would in turn increase the epidermal penetration rate and reduce the dermal clearance, thus resulting in the attainment of higher concentrations in the viable epidermis.

The topical efficacy of methotrexate could probably be increased by reducing its dermal clearance (Chapter 7). This might be achieved by combining methotrexate with a steroid, at a concentration sufficient to cause vasoconstriction, which may result in attainment of concentrations in the viable epidermis capable of inhibiting the epidermal DNA synthesis necessary to maintain psoriasis. On the other hand this might prove to be a futile approach, as in psoriasis the structural alterations in the stratum corneum and the dermis are likely to significantly increase the overall rate of disappearance of methotrexate from the site of the disorder (Chapter 7). This means that even the presence of vasoconstriction may not suffice, as the dermal clearance, might remain high following morphological change in the dermis.

The <u>in vitro</u> penetration of weak electrolytes with and without

iontophoresis (Chapters 5 and 6) show that it may be possible to administer weak electrolytes, especially new compounds, even if the substance is kept in a highly ionised form. The possible penetration of both ionised and unionised species through the human stratum corneum indicate that the extent of ionisation of the drug in a dosage form used for either local or systemic effect, may not be an important criterion. The mechanism of penetration of weak electrolytes in form of ion-pairs shows that the percutaneous penetration of ionised species may be enhanced by the use of an appropriate counter ion (Chapter 5), which is capable of promoting the transport of ionised species through the human stratum corneum.

Iontophoresis is a well documented method whose potential use in administering drugs topically for local or systemic effect has only been realized in last decade (Chapter 6; Macek 1983). The in vitro results obtained in the present work suggest that if a weak electrolytes shows poor percutaneous penetration, it is possible to administer that drug by using iontophoresis provided the drug was kept in a highly ionised form (Chapter 6). The use of iontophoresis is not limited to weak electrolytes, as it has been previously suggested that even non-electrolytes could be administered by iontophoresis. This technique is referred to as "iontohydrokinesis", which uses ions (Na or Cl) in aqueous solution to administer the non-electrolyte drug by facilitated transport (Gangarosa et al 1980).

The possible penetration of ionised species or even steroids through the "intracellular" route (Chapters 4, 5 and 6) indicates

that the site of topical administration of a drug (section 2.3.2) may not be an important consideration, however the presence of hair follicles at the site of administration of the drug may increase the overall rate of penetration by a small amount.

Future work

Various aspects of the topical administration of drugs, for either local or systemic effect, have been the subject of numerous investigations (Chapters 1 and 2). These *in vitro* and *in vivo* investigations have helped in the development of an understanding of the importance of many factors and in the development of topical dosage forms, which are now used to treat both local systemic disorders (Chapter 1).

Some aspects of the present work should be investigated further; for example the importance of molecular structure. The effect of different vehicles (section 2.3.1.4) on the mechanism and the rate of *in vitro* and *in vivo* penetration of steroids and weak electrolytes (with and without iontophoresis) could also be investigated.

The development of the use of iontophoresis to administer large molecules (e.g. hormones) topically has great potential for the future and should be the subject of much more intense investigation than has been evident up to the present time.

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APPENDICES

Appendix: 1

Compartmental model I: The compartmental model I (CM I) used during this work was the equation 15 of Wallace and Barnett (1978) which was reported as:

$$A_3 = J_s (t - \tau + e^{-E_2 t}) \qquad \text{Eq. A1.1}$$

where J_s = flux, τ = lag time and E_2 = exponential coefficient.

Equation A1.1 can also be expressed as equation 5.12 after substituting the relevant parameters reported by Wallace and Barnett (1978) in the above equation.

Appendix: 2

Derivation of compartmental model II: The rate equations for compartmental model II (CMII, Figure 5.1b) can be written as:

$$-\frac{dA_2^u}{dt} = k_{23}^u A_2^u - k_{12}^u A_1^u \qquad \text{Eq. A2.1}$$

$$-\frac{dA_2^i}{dt} = k_{23}^i A_2^i - k_{12}^i A_1^i \qquad \text{Eq. A2.2}$$

$$\frac{dA_3}{dt} = k_{23}^i A_2^i + k_{23}^u A_2^u \qquad \text{Eq. A2.3}$$

Using Laplace transformation, equations A2.1-A2.3 can also be written as the following set of simultaneous equations:

$$-S\bar{A}_2^u = k_{23}^u \bar{A}_2^u - k_{12}^u A_{1/S}^u \qquad \text{Eq. A2.4}$$

$$-S\bar{A}_2^i = k_{23}^i \bar{A}_2^i - k_{12}^i A_{1/S}^i \qquad \text{Eq. A2.5}$$

$$S\bar{A}_3 = k_{23}^i \bar{A}_2^i + k_{23}^u \bar{A}_2^u \qquad \text{Eq. A2.6}$$

Solving for the amount "A_3" in the receptor compartment (CMII, Figure 5.1b). A_1^u and A_1^i were divided by S to convert amounts into concentrations. Therefore after substitution and rearrangements of equations A2.4, A2.5 and A2.6, one can obtain the following:

$$\bar{A}_3 = \frac{A_1^i k_{23}^i k_{12}^i}{S^2(S+k_{23}^i)} + \frac{k_{23}^u k_{12}^u A_1^u}{S^2(S+k_{23}^u)} \qquad \text{Eq. A2.7}$$

Equation A2.7 is retransformed into F(t) using the Laplace table, as:

$$M = \frac{C_i k_{23}^i k_{12}^i}{k_{23}^i} t - \frac{C_i k_{23}^i k_{12}^i}{(k_{23}^i)^2}\left(1 - e^{-k_{23}^i t}\right)$$

$$+ \frac{C_u k_{23}^u k_{12}^u}{k_{23}^u} t - \frac{C_u k_{23}^u k_{12}^u}{(k_{23}^u)^2}\left(1 - e^{-k_{23}^u t}\right) \qquad \text{Eq. A2.8}$$

Equation A2.8 can be further simplified to:

$$M = C_i k_{12}^i t - \frac{C_i k_{12}^i}{k_{23}^i}\left(1 - e^{-k_{23}^i t}\right)$$

$$+ C_u k_{12}^u t - \frac{C_u k_{12}^u}{k_{23}^u}\left(1 - e^{-k_{23}^u t}\right) \qquad \text{Eq. A2.9}$$

In equation A2.9 it is assummed that A_1 is in contact with the stratum corneum and is expressed in terms of concentration, $A_1 = C*V$.

Appendix: 3

Goodness of fit for the models: An analysis of variance ($P < 0.01$) was carried out to compare the unweighted residual sum of squares (RSS) differences between the fits of the diffusion models (DM I or III) by the F-test (Boxenbaum et al 1974), used to describe the *in vitro* permeation of weak electrolytes through the human stratum corneum. A F-test was also done on compartmental model used for methotrexate.

A3.1 Methotrexate: N = 72, DM I-RSS = 0.6680, DM III-RSS = 0.1020, CM I-RSS = 0.6678, CM II-RSS = 0.1064.

A3.1.1 DM I v/s DM III - A significant difference was found between the models ($F = 189$, df = 2/68).

A3.1.2 CM I v/s CM II - A significant difference was found between the models ($F = 179$, df = 2/68).

A3.2 Salicylic acid: N = 29, DM I-RSS = 0.0016 and DM III-RSS = 0.00086.

A3.2.1 DM I v/s DM III - A significant difference was found between the models ($F = 10$, df = 2/25).

A3.3 Aspirin: N = 30, DM I-RSS = 0.0015, DM III-RSS = 0.000041.

A3.3.1 DM I v/s DM III - A significant difference was found between the models ($F = 463$, df = 2/26).

A3.4 Lignocaine hydrochloride: N = 36; DM I-RSS = 0.142, DM III-RSS = 0.0069.

A3.4.1 DM I v/s DM III - A significant difference was found between the models (F = 313, df = 2/32).

A3.5 Chlorpromazine hydrochloride: N = 35, DM I-RSS = 0.000028, DM III-RSS = 0.0000037.

A3.5.1 DM I v/s DM III - A significant difference was found between the models (F = 102, df = 2/31)

A3.6 Chlorpheniramine maleate: N = 38, DM I-RSS = 0.00059, DM III-RSS = 0.000025.

A3.6.1 DM I v/s DM III. A significant difference was found between the models (F = 384, df = 2/34).

A3.7 Ephedrine hydrochloride: N = 36, DM I-RSS = 0.169, DM III-RSS = 0.035.

A3.7.1 DM I v/s DM III. There was a significant difference between the models (F = 61, df = 2/32).

A3.8 Pilocarpine hydrochloride: N = 26, DM I-RSS = 0.007, DM III-RSS = 0.005.

A3.8.1 DM I v/s DM III. There was a significant difference between the models (F = 3.14, df = 2/22).

It was not possible to compare DM III and CM II using the F-test method due to the number of parameters being the same for both the models.

Appendix: 4

An analysis of variance ($P < 0.05$) of the data of weak electrolytes was carried out to determine the dependence of steady state flux (linear regression) and the lag times on the pH of the aqueous solution (section 3.7.5).

A4.1 Methotrexate: $F = 1032$, $df = 4/10$.

A4.2 Salicylic acid: $F = 1495$, $df = 4/10$.

A4.3 Aspirin: $F = 581$, $df = 4/10$.

A4.4 Lignocaine hydrochloride: $F = 296$, $df = 5/12$.

A4.5 Chlorpromazine hydrochloride: $F = 11483$, $df = 5/12$.

A4.6 Chlorpheniramine maleate: $F = 350$, $df = 5/12$.

A4.7 Ephedrine hydrochloride: $F = 6999$, $df = 5/12$.

A4.8 Pilocarpine hydrochloride: $F = 860$, $df = 1/4$.

The F-values of the above substances indicate that the steady state flux and lag times were pH dependent.

Appendix: 5

An analysis of variance and a test of least significant difference (lsd) was carried out to compare the steady state fluxes (linear regression) with and without iontophoresis (section 3.7.5).

A5.1 Methotrexate: There was a significant difference in fluxes with and without iontophoresis which was dependent on the degree

of ionisation ($F = 1034$, $df = 4/10$, $P < 0.05$) for pH values 3.4 to 8.0 (lsd, $P < 0.05$).

A5.2 Salicylic acid: There was a significant difference in fluxes with and without iontophoresis which was dependent on the degree of ionisation ($F = 1494$, $df = 4/10$, $P < 0.001$) for pH values 3.6 to 8.0 (lsd, $P < 0.005$).

A5.3 Aspirin: There was a significant difference in fluxes with and without iontophoresis which was dependent on the degree of ionisation ($F = 581$, $df = 4/10$, $P < 0.001$) for pH values 3.4 to 8.0 (lsd, $P < 0.05$).

A5.4 Lignocaine hydrochloride: There was a significant difference in fluxes with and without iontophoresis which was dependent upon degree of ionisation ($F = 295$, $df = 5/12$, $P < 0.001$). A test of lsd showed that between pH values of 3.4 and 8.0 there is evidence of significant difference in fluxes with and without iontophoresis (lsd, $P < 0.05$) whereas at pH values of 9.4 and 11.7 the change in flux due to iontophoresis is not significant (lsd, $P > 0.05$).

A5.5 Chlorpromazine hydrochloride: There was significant difference in fluxes with and without iontophoresis which was dependent on degree of ionisation ($F = 11483$, $df = 5/12$, $P < 0.001$). A test of lsd showed that between pH values of 3.6 and 7.2 there is evidence of significant difference in fluxes with and without iontophoresis (lsd, $P < 0.05$) whereas at pH values of 8.4 and 11.7 the change in flux due to iontophoresis is not significant (lsd, $P > 0.05$).

A5.6 Chlorpheniramine maleate: There was a significant difference in fluxes with and without iontophoresis which was dependent on degree of ionisation ($F = 349$, $df = 5/12$, $P < 0.001$). A test of lsd showed that between pH values of 3.4 and 9.4 there is evidence of significant difference in fluxes with and without iontophoresis (lsd, $P < 0.05$) whereas at pH value of 11.7 the change in flux due to iontophoresis is not significant (lsd, $P > 0.05$).

A5.7 Ephedrine hydrochloride: There was significant difference in fluxes with and without iontophoresis which was dependent on degree of ionisation ($F = 6999$, $df = 5/12$, $P < 0.001$). A test of lsd showed that between pH values of 3.6 and 8.0 there is evidence of significant difference in fluxes with and without iontophoresis (lsd, $P < 0.05$) whereas at pH values of 9.6 and 11.7 the change in flux due to iontophoresis is not significant (lsd, $P > 0.05$).

A5.8 Pilocarpine hydrochloride: There was a significant difference in fluxes with and without iontophoresis which was dependent on degree of ionisation ($F = 870$, $df = 1/4$, $P < 0.001$). A test of lsd showed that at pH values of 3.6 and 8.0 there is evidence of significant difference in fluxes with and without iontophoresis (lsd, $P < 0.05$).